RNI No. ORI ENG / 2003 / 15643 / 22.11.2005

ISSN No. 0974 - 4398 / 2008

e-planet

Volume - 14 December - 2016 Issue No. - 2

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

Editorial Board

Printed, published & edited by Dr. R.K. Samantaray, 1(A), Nandankanan Zoo, P.O. Barang, Dist- Khurda, Odisha, India, Pin - 754005. Owned by the Organisation for Protection of Ecosystem, Environment and Endangered Species (OPES), Plot No. - A-47, Rameswarpatna, Maushima Square, Old Town, Bhubaneswar-751002, Odisha, India. Published and printed at Print-Tech Offset Pvt. Ltd., Bhubaneswar - 751024

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e-planet

Volume - 14 **December - 2016** **Issue No. - 2**

A Multi-disciplinary International Journal

of

Ecology, Environment, Agriculture and Allied Sciences

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e-planet **14** (2): 1-9 (December 2016)

Important fungal diseases of rice and their integrated management

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Date of receipt: 07. 11. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

Rice is the most important staple food consumed by nearly half of the world population in Asian regions. It is found that the losses in rice crop production are caused due to insects, diseases, weeds and vertebrate pests. The serious affection is mostly due to different fungal diseases. It has been revealed that the most dreaded fungal diseases of rice are blast and sheath blight apart from others such as brown spot, sheath rot, false smut, leaf scald, stem rot, grain discoloration and udbatta. In recent years, the need to intensify rice production to feed a rapidly expanding population, we need to adopt the integrated management practices of the aforesaid diseases. The fungal diseases can be managed through the biological control agents, use of mechanical and chemical practices, selective fungicides, cultural practices and botanical products as safer alternatives to pesticides. This review paper gives an insight on occurrence and spread of the fungal diseases and the adoption of different measures towards their prevention and control to safeguard the growing demand of rice both in terms of production and productivity.

Key words: Fungal diseases, integrated disease management (IDM), bio-control agents, botanicals

INTRODUCTION

Rice, the queen of cereals, is the most important crop of India. The saying 'Rice is life' is most appropriate for India as this crop plays a vital role in our national food security and is a means of livelihood for millions of rural households. To feed the ever growing population per annum in India, a compulsive increase in crop yield by accelerating cropping intensity has become an immediate inevitable option. However, intensive cultivation unfortunately makes it more congenial for disease, pest population. Apart from some serious environmental and logistic problems, this crop is subjected to the serious ravages of different diseases causing a major setback in achieving the right production. As many as 40 diseases of rice in India have been reported to be caused by different fungi and bacteria (Padwick, 1950) and subsequent additions to the list, of late, the number of diseases have increased to 60 (Chakrabarti, 1980). Important rice diseases are caused by the bacteria, fungi and viruses. Among the fungal diseases, rice blast and sheath blight are more devastating and wide spread. Other fungal diseases like brown spot, sheath rot, false smut, leaf scald, *bakanae*/foot rot, stem rot, glume/grain discoloration and udbatta are also serious at certain locations. These diseases cause significant damage to the grain and straw yield. To avoid yield losses

caused due to different fungal diseases, an appropriate and comprehensive management strategy must be combined together and adopted.

IMPORTANT FUNGAL DISEASES OF RICE

Details of some of the important fungal diseases of rice along with their integrated management are discussed below.

Blast: *Pyricularia grisea* Saccardo (Perfect stage: *Magnaporthe grisea*)

Rice blast, otherwise called as 'rice fever disease', is a severe fungal disease which occurs during both *kharif* (wet) and *rabi* (dry/summer) season. The name 'blast' denotes quick death of a part or entire plant. Blast disease is generally considered as the principal disease of rice because of its wide distribution and destruction under favourable conditions. The loss due to this disease has been reported from 1.4% to 100%. Due to high variability in virulence of this pathogen, resistant variety of one location may become highly susceptible at the other location.

Typical spindle shaped leaf lesions, wide at centre and pointed towards either end are observed. In severe cases, they coalesce and the leaves die. Neck and panicle infection cause chaffy and shriveled grains. The node becomes black and breaks at the joint.

Panicles dry up and break down before harvest. Predisposing factors for the outbreak of this disease are: low night temperature of 20-24°C with RH 90% or above; light showers of rain continuing for few days or cloudy weather persisting for few days; acidic soils with pH of 5.0-6.5; copious dew formation occurring in susceptible stages of the crop; application of high dose of N-fertilizers (>80 kg N ha^{-1}). Sowing of seeds in nursery beds in the upland or direct sowing in the upland favour the development of this disease.

Resistance to *Magnaporthe oryzae* is known to follow gene for gene theory, where major resistance (R) gene is effective in preventing infection by a race of *M. oryzae* containing the corresponding avirulence (*Avr*) gene. So far, over 100 blast resistance genes have been identified, of them, 45% are from *japonica* cultivars, 51% are from *indica* cultivars and the remaining 4% are from wild species of rice. These R genes are distributed all over the 12 rice chromosomes except chromosome 3. Out of them, 23 have been cloned and characterized *viz*., *Pib, Pita, Pik-h, Pi9, Pi2, Piz-t, Pid2, Pi36, Pi37, Pik-m, Pit, Pi5, Pid3, pi21, Pb1, Pish, Pik, Pik-p, Pia, NLS1, Pi25, Pi54rh* and *Pi64*. The majority of the blast R genes are dominant and qualitative, except the recessive gene *pi21*, and a few *pi21* and *Pb1*being reported as quantitative in nature.

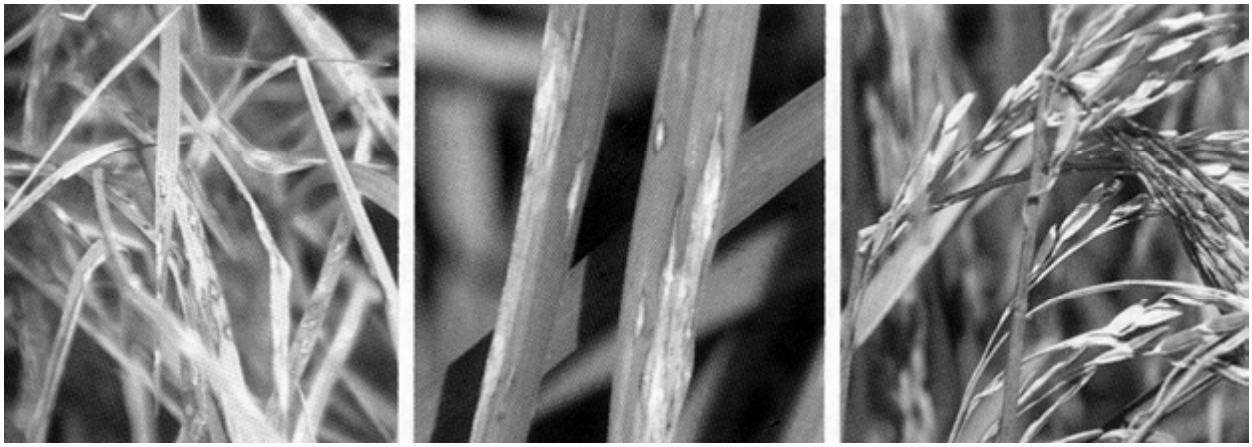


Fig. 1 **Fig. 2** **Fig. 3**

(Fig. 1. Blast symptoms in leaves, **Fig. 2.** Congregation of blast symptoms in leaves, **Fig. 3.** Neck blast symptoms)

Brown spot (sesame leaf spot): *Helminthosporium oryzae* (perfect stage: *Cochliobolus miyabeanus*)

It is a fungal disease which generally appears on the leaves. The fungus was first described by Breda de Haan in 1900. Losses due to this disease in seedling mortality has been reported from 10-58% and during the great Bengal famine losses have been reported to be as high as 80% (Padmanabhan, 1977). Typically ellipsoidal, oval to circular lesions are seen on the coleoptiles, leaf blade, leaf sheath and glume, measuring about 2-8 mm x 0.5 mm which coalesce in severe infections. Light brown grey centre with dark or reddish brown margin, blackened grains, characteristic burnt and scorched appearance of the field in advance stage are the identifying symptoms.

Some of the factors which favour spread of this disease are: occurrence of disease in severe form either at high or low N level; plants grown in soil deficient in K, Ca, Mg and Zn; occurrence of heavy rainfall in September accompanied by temperature of 25-30^{o}C followed by continuous cloudy weather in October to November; luxuriant growth of plants etc. The disease also becomes prominent due to poor status of soil or unfavourable soil conditions or due to imbalanced fertilizer input or iron toxicity and it is both seed and air borne. The *bora* (glutinous) rice is more often affected by brown spot as compared to non-glutinous rice. For example, grains of Aghoni *bora* variety are badly affected by this disease.

Fig. 4. Brown spot symptoms appear on the leaves

Narrow brown leaf spot (*Cercospora* leaf spot): *Sphaerulina oryzina* (syn. *Cercospora oryzae*)

This disease infects leaves, sheaths, and panicles. It leads to premature death of leaves and leaf sheaths, premature ripening of grains and in severe cases leads to lodging of plants. Typical lesions on leaves and upper leaf sheaths are light to dark brown, linear and progress parallel to the vein. They are usually 2"10 mm long and 1"1.5 mm wide. Generally it is observed in potassium deficient soils and in areas with temperature ranging from 25- 28°C. Severe damage due to this disease reduces the market value of the grains and the milling recovery.

Sheath blight: *Rhizoctonia solani* Kuhn [Perfect stage: *Thanatephorus cucumeris* (Frank) Donk]

It is also a serious fungal disease, observed to become more prevalent in many HYVs of rice in low altitude areas, appearing late in the season but quite severe in *kharif* season. A modest estimation of losses due to sheath blight in India has been reported up to 54.3% (Rajan, 1987 and Roy, 1993). The disease affects all plant parts above the water line, *viz.*, sheath, inter-nodes, upper leaves and panicles. The symptoms first appear on the lower leaves near the water level during tillering stage with lesions which are oblong or elliptical, greenish grey in colour, water soaked (about 1 cm in length). These lesions have regular dark brown and grayish central region. The infected leaves and internodes turn grey to straw colour with lateral brown bands resembling snake skin. Spherical, dark brown sclerotia of 3-5 mm appear in the affected area. Favourable factors like optimal temperature of 28-34^{o} C with high Relative Humidity(RH) 95%, application of higher dose of N fertilizers (> 80 kg N ha^{-1}), early sowing and early transplanting, close canopy and presence of root knot nematode help in spread of this disease significantly.

The pathogen has a wide host range (grasses, other weeds on bunds of rice field being carrier of this disease). It is both soil and seed borne. *R. solani* is divided into strains or varieties called Anastomosis Groups (AG). The strain on rice is called AG1- 1A. It doesn't produce any spores, therefore, being called as a sterile fungus in India.

Fig. 5

Fig. 6

Fig. 7

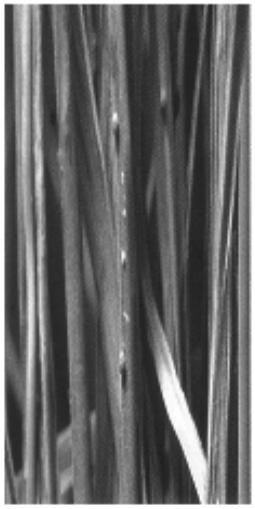


Fig. 8

(Fig. 5. Sheath blight lesion on rice stem, **Fig. 6.** Banded sheath blight symptoms, **Fig. 7.** Severely affected rice plants in fields, **Fig. 8.** Sclerotia on the sheath blight affected rice plant parts)

Sheath rot: *Sarocladium oryzae*

It is a wide spread disease in Japan, very common in S.E Asia and the Indian subcontinent. Damage caused by this disease is reported to be moderate in irrigated and favourable low land rice fields. This disease appears during heading to maturity stages and the pathogen generally attacks the uppermost leaf sheath enclosing the young panicles. Oblong or irregular spots, 0.5 to 1.5 cm long with brown margins and grey centres with chocolate brown colouration develop on the boot leaf sheath. They enlarge, often coalesce and may cover most of the leaf sheath. If the infection comes before panicle emergence, the panicle may not emerge or rot completely or emerge partially with rotten grains. If it comes after panicle emergence, the grains are partially filled with discoloured glumes. Severe infection causes poor exertion of panicles and grains. Disease progress leads to sterility of panicles.

Fig. 9

Fig. 9. Sheath rot disease of rice, *Sarocladium oryzae*

Fig. 10

Fig. 10. Grains in the panicle partially/ fully enclosed in the boot leaf

False smut: *Ustilaginoidea virens* (Perfect stage: *Claviceps oryzae-sativae*

This disease was first reported by Cooke (1978). It is called as false smut as it is not a true smut. A true smut like loose smut in wheat belongs to the sub-division, Deuteromycotina (conidial stage) or Ascomycotina (ascigerous stage). The disease symptom is visible only after panicle emergence at milk stage. A few grains in the panicle are transformed into a mass of spores that are greenish outside and yellow orange on the inside. The spore balls are found in between glumes measure 1cm and longer when mature. The grains next to the spore balls are empty. Chlamydospores cannot be easily freed from the smut balls because of the presence of a sticky material. Weather parameters namely, low temperature (20°C),high relative humidity (>92%), moderate rainfall with intermittent clear and drizzling weather during the period from flowering to maturity favour the spread of this disease. Many grasses and wild rice are the sources of inoculum and mode of transmission of this disease is through the air.

Stem rot: *Helminthosporium sigmoideum* (Perfect stage: *Magnaporthe salvinii*)

The fungus is most commonly found in its sclerotial stage, *Sclerotium oryzae,* Cattaneo and also in the conidial stage, *Nakataea sigmoidea* (Cavera) Subramanian. Infection due to this disease occurs usually near the waterline, entering through the wounds and injuries. Disease symptoms are generally observed in the rice plants after mid-tillering stage, as blackish, dark, irregular lesions on the outer leaf sheath and gradually enlarges. The fungus penetrates into the culm, weakens the stems and causes lodging.

Udbatta: *Ephelis oryzae*

This disease is observed mainly in the hilly regions and can be detected only at heading stage of the crop growth. The boot leaf of the affected plant is slightly distorted and a silvery lusture is observed on the upper surface of the leaf blade. Instead of a normal branched panicle, a reduced, compact rod like panicle of grey to black colour is produced. This disease is seed borne.

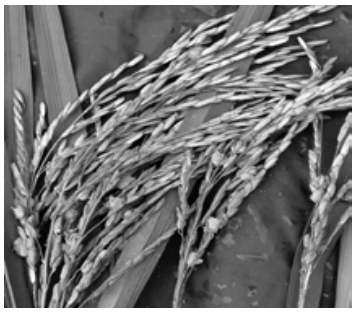


Fig. 11

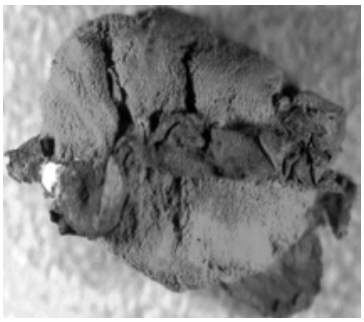


Fig. 12

Fig. 13

(Fig. 11/ 13. False smut balls in the affected panicles, **Fig. 12.** Enlarged view of the smut ball)

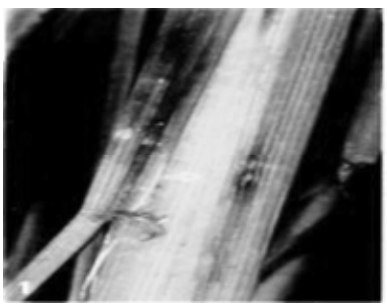


Fig. 14

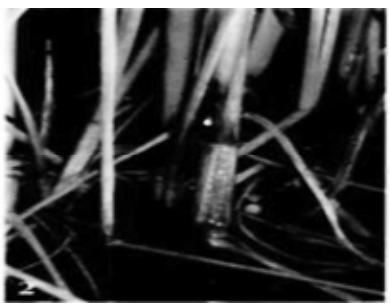


Fig. 15

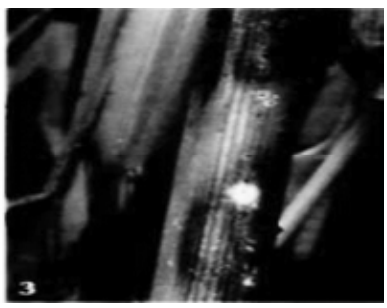


Fig. 16

(Fig. 14, 15 and 16: Stem rot symptoms**)**

INTEGRATED MANAGEMENT OF FUNGAL DISEASES

Integrated disease management (IDM) aims to combine multidisciplinary approaches and to develop disease management strategies practical, effective, economical, protective of both public health and environment. IDM lies in the suitably tailored cultural practices, judicious management of resistant genes in rice cultivars, use of chemicals/ botanical based products on the basis of surveillance.

After careful analysis, the following integrated management strategies are recommended. It has been recommended to grow resistant and tolerant rice cultivars (Table 1).

Table 1. Growing resistant/ tolerant varieties of rice cultivars against fungal diseases

Fungal diseases	Resistant/ tolerant cultivar
Blast	IR 20, IR 36, IR 42, IR 64, Rasi, Vandana, ADT 30, Ranjit, Bahadur, Ketekijoha, Jalashree, Samalei, Lakhimi, Seema, Parijat, Gayatri, Hazaridhan, Chandan, Salivahan, Ajaya, Rajalaxmi, Dharitri, Heera, Jaya, Jitendra, Krishna Hamsa, Nidhi, Pankaja, Pusa Basmati 1, Pusa Suganha 2, Pusa Sugandha 3, Ratna, Savitri, Pooja, , Tulasi, Vikramarya, Bahadur, Chandrama, Luit, Manoharsali, Pratikshya
Brown spot	Rasi, Jaganath, IR 36, Phalguni, Sahabhagidhan,Virendra, CR Dhan 40, Sadabahar,Vandana,CR Dhan 601(Boro), Abhishek, Saket 4,Reeta
Sheath blight	Pankaj, CR 1014, Ratna, Vikramarya, IR 64, Seema, Nalini, Manohar Sali, Lakhimi, Sabita,Seema, Matangini, Neela, Indira-, Chandrama, Satabdi, Reeta, Ketekijoha, CR 1002,Hanseswari
Sheath rot	Bala, Cauvery, Sabarmati, Swarnadhan, Phalguna, Vikramarya, Phalguni, Sahabhagidhan, Anjali, CR Dhan 601, Satyakrishna, Satabdi
False smut	Gayatri, Savitri, Panidhan, Sitabhog, Mayurkantha, Pankaj, Cauvery, Sabarmati, Pankaj, Bala, Sabarmati, Parijat,Vijaya
Stem rot	Jaganath, Sabarmati, Pankaj, Givind, Jalamagna, Basmati 3, Basmati 370, Mushkan 7, Mushkan 41

Cultural practices

- Deep summer ploughing followed by flooding of field
- Trimming of bunds and destruction of crop residues, weed hosts etc.
- Selection of resistant/ moderately resistant/ tolerant varieties
- Use of healthy seeds from disease free crop.
- Early and timely sowing/ transplanting
- Raising of healthy nursery
- Practising wider nursery
- Avoidance of close spacing/ dense planting and use of excess of fertilizers
- Use of cow dung based Farm Yard Manure (FYM).
- Proper water management practices
- Harvesting of the produce close to the ground level
- Practice of green manuring with dhaincha.
- Application of neem cake in the main field @ 2.5 q ha^{-1}.
- Hot water/ salt water seed treatment
- Treat the seeds with Carbendazim 50WP@ 2g kg^{-1} seeds before sowing to avoid seed borne diaeses.
- Seed treatment with the biocontrol agent, *Trichoderma viride* 1WP @ 4g kg^{-1} or *Pseudomonas fluorescens* 0.5 WP@10g kg^{-1} of seed
- Removal of weeds and weedy rice

Mechanical practices

Removal and destruction of disease affected plant parts may be practised if there is low disease intensity of rice plants, so as to inhibit the spread of the diseases.

Chemical control

- Spray with Tricyclazole 75 WP @ 0.6g l^{-1} of water or Carbendazim 50WP @1g l^{-1} of water for control of blast disease.
- Spray with Propiconazole 25 EC @ 1ml l^{-1} or Mancozeb 75 WP @ 2g l^{-1} or Carbendazim 50WP @1g l^{-1} against brown spot disease.
- Give need based spray of effective fungicides- Validamycin 3L @ 2.5ml l^{-1} or Hexaconazole 5EC @ 2ml l^{-1} or Thifluzamide 24SC@1ml l^{-1} or Carbendazim 50WP @1g l^{-1} against sheath blight of rice.
- Spray twice at 10days interval starting from boot leaf stage with Carbendazim 50WP @ 1g l^{-1} for control of sheath rot disease.
- For control of false smut disease, spray Carbendazim 50WP @1g l^{-1} or Mancozeb 75WP@ 2g l^{-1} or Carbendazim 12% + Mancozeb 63% WP @ 1.5g l^{-1} or Copper hydroxide 77WP @ 2g l^{-1} of water.

The economic threshold levels for different diseases in rice are directly proportional to the incidence of diseases at different stages of crop growth (Table 2).

Table 2: Economic threshold levels identified for different diseases in rice

Diseases	Crop growth stages		
	Seedling	Tillering	Flowering and after
Leaf blast (% leaf area)	5%	5-10%	5-10%
Brown spot	-	2-5%	2-5%
Sheath blight	-	10% or more	10% or more
Sheath rot (% tillers affected)	-	-	2-5%

Biological control practices

Bio-control agents/ antagonists are considered as one of the effective and eco-friendly means of management of diseases in different crops. A number of bio-control agents like *Pseudomonas fluorescens*, *Bacillus* spp. against blast and *Trichoderma viride*, *T. harzianum*, *P. fluorescens*, *Gliocladium virens*, *Aspergillus terreus* against sheath blight have been found effective in reducing the disease incidences, increasing production and productivity.

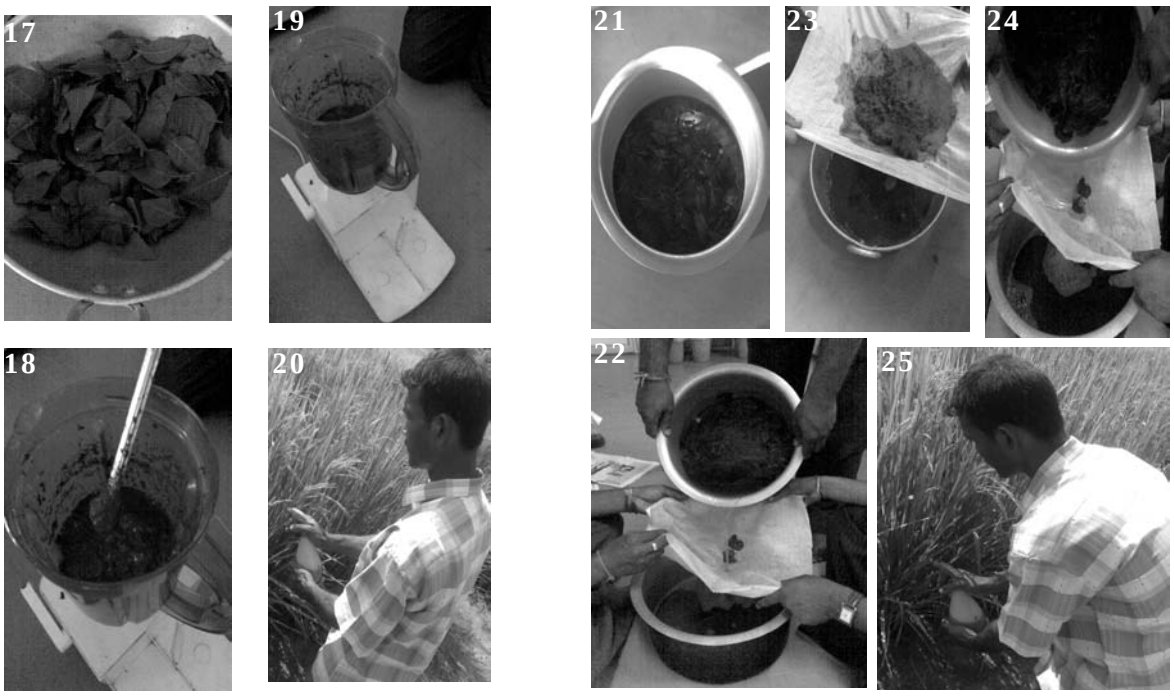
Method and dose of application of bio-control agents

For control of sheath blight disease in rice, the bio-control agent *T. viride* is to be applied in the main field before transplanting with rice plants by (i) mixing 1 kg of the formulation with 25 kg FYM ac^{-1} and broadcasting, (ii) Foliar spray with 5 gm l^{-1} of water.

Another bio-control agent, *Pseudomonus fluorescens* can be sprayed in rice plants affected with sheath blight disease @ 3 gm of formulation per litre of water. Research findings revealed that *T. viride* was the most effective bio-control agent followed by *P. fluorescens* which is *at par* with the standard fungicide, validamycin 3% l (Sheathmar-3).

Botanicals as a safer alternative to pesticides

- Certain constraints have been experienced often in use of synthetic chemicals to control the disease i.e., blast due to escalating the cost of the fungicides, non-availability at the time need, increasing resistance to blast pathogen due to repeated use, residual toxic effect and environmental pollution.
- During the last three decades several reports have been documented on botanical products producing antifungal, antibacterial or antiviral substances (Ramakrishnan, 1948; Tewari, 1995 and Tewari and Patra, 2006).
- Various preparations from plants such as aqueous/ steamed aqueous, ethanol petroleum ether, hexane, benzene, chloroform methanol, essential oil or pure active ingredients such as limonene, eugenol or thiamine have been tried against rice blast pathogen in the laboratory at Central Rice Research Institute, Cuttack (Tewari and Mishra,1990; Tewari and Nayak, 1991; Tewari, 1995 and Mishra et al., 1997).
- Keeping these above factors in view, the performance of two botanicals namely bael and tulasi were evaluated and compared with the standard fungicide Ektino (Tricyclazole 75 WP for their efficacy and ability in controlling blast disease of rice.
- The botanical products developed from bael, *Aegle marmelos* Corr @ 25 green leaves l^{-1} of water (by grinding to a fine paste) and tulasi, *Ocimum sanctum* L.@ 25gm green leaves l^{-1} of water (on boiling) can be used effectively in managing the blast disease of rice.
- The research findings as obtained from the experiments conducted at CRRI, Cuttack, Odisha showed reduction in blast incidence significantly due to treatment with bael followed by tulsi, being at par with the standard fungicide, Tricyclazole 75 WP.



(**Fig. 17, 18, 19, 20.** Stages of preparation of bael leaf extract and its application in the rice field)

(**Fig. 21, 22, 23, 24, 25.** Stages of preparation of tulsi leaf extract and its application in the rice field)

CONCLUSION

Fungal diseases are one of the major biotic constraints to rice cultivation causing severe yield losses. There are ample possibilities of improving rice production and productivity through an integrated fungal disease management strategy by combining cultural practices, mechanical practices, host plant resistance, need based application of chemicals, eco-friendly use of bio-control agents and botanical products. High yielding varieties of rice having notable resistance/ tolerance to the fungal diseases need to be developed/ improved for the targeted crop production/ productivity.

There is also a need to develop effective virulent strains of bio-control agents. Research on botanical pesticides especially identification of their active components, developing their active formulations and their evaluation against the target fungal diseases and also validation of the botanicals being used under indigenous technical knowledge (ITK) may be strengthened.

The chemical pesticides should be used judiciously without disturbing the ecological balance and causing no harm to the beneficial micro-organisms.

Disease control measures (for fungal diseases) have to be focused on resistant/ tolerant genotypes, local adaptability and more virulent bio-control agents. Reconstruct of Integrated Disease Management (IDM) modules are required having integrated and holistic approach using minimum, but cost effective interventions. This approach will not only improve the crop productivity, but also minimize the cost towards disease management.

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e-planet **14** (2): 10-19 (December 2016)

Selenium yeast: An organic source of selenium for animals

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Date of receipt: 04. 05. 2016 Date of acceptance: 26. 12. 2016

ABSTRACT

Selenium is physiologically essential for animals and human beings and may also offer a protective effect against several diseases. The organic form of selenium provided by selenium yeast has been shown to differ in bioavailability and metabolism compared with inorganic (selenate and selenite) forms of dietary selenium. Selenium yeast, produced by fermented *Saccharomyces cerevisiae* in selenium enriched media, is a recognized source of organic form of selenium. Dietary supplementation using selenium yeast has been associated with increased ability to counteract oxidative stress and enhanced immune status, growth and reproduction of animals. The consequent improvements in productivity can be of economic benefit to livestock producers for many reasons, including greater overall efficiency of feedstuff use. Selenium yeast supplementation in animal diets has an added nutritional benefit to human consumers. Dietary supplementation of selenium yeast enhanced the selenium content in meat, milk and egg, consequently producing selenium-rich functional foods like selenium enriched eggs and meats for human consumption.

Key words: Animals, functional food, selenium yeast, dietary supplement

INTRODUCTION

Selenium (Se) is a trace element with atomic number 34 and atomic weight 78.96 belonging to group VI of the periodic table. It is toxic at high doses and deficiency is a global problem related to an increased susceptibility to various diseases of animals and humans and decreased productive and reproductive performance of farm animals. The nutritional essentiality of Se arose from the work of (Patterson et al., 1957) in chicks. Se plays an important role in numerous biochemical functions such as antioxidant defense, immune function, reproduction, and thyroid hormone metabolism (Surai, 2002). Several diseases in cattle are caused by deficiency of Se. Such conditions include nutritional muscular dystrophy (white muscle disease), retained fetal membranes, increased susceptibility to mastitis, infertility, abortion, premature birth, weak or dead calves, cystic ovaries, metritis, delayed conception and poor fertility (Spears et al., 1986). Se is also a component of enzyme type I deiodinase (IDI), which is required for the conversion of thyroxine (T_4) into more active tri-iodothyronine-T_3 (Beckett et al., 1987). Se has also been shown to improve immune responses in animals (Reddy et al., 1987). Vitamin E and Se supplementation are usually combined since they both exert complementary anti-oxidant activities.

HISTORY

Se was discovered by Jons Berzelius in 1817 as a contaminant of sulphuric acid vats that caused illness in Swedish factory workers. But its beneficial role in animal nutrition begins in 1957 with the finding that a factor (Factor 3) in yeast would prevent liver necrosis in rats (Schwarz and Foltz, 1957). In the 1970s, it was discovered to be an essential cofactor of the enzyme glutathione peroxidase (Schwarz, 1976).

In nature, Se exists in two chemical forms, inorganic and organic. In particular, inorganic Se can be found in different minerals in the form of selenite ($SeO(OH)_2$), selenate ($SeO_2(OH)_2$) as well as in the metallic (SeO) form. Organic forms come mainly from the decomposition of plants that accumulate Se (Martens and Suarez, 1996). In contrast, Se in feed ingredients (forages, grains, oilseed meals *etc.*) is an integral part of various organic compounds including amino acids selenomethionine (SeMet) and selenocysteine (SeCys) and exists in the Se^{-2} oxidation state. As a result, in nature animals receive Se mainly in the form of SeMet which is considered to be a most effective nutritional form of Se for animals and human. In fact SeMet fulfils the criteria of an essential amino acid (Schrauzer, 2003).

SELENIUM IN PROTEINS

Se is present in protein in the forms of either SeCys or SeMet residues. SeMet of exogenous sources can be incorporated in its intact form into proteins by the Met codon without distinguishing between Se-Met and Met i.e. AUG codon (Butler and Whanger, 1989). The codon for SeCys incorporation is UGA that is stop codon in general. Diverse inorganic and organic Se compounds are converted to selenide or its equivalent, which is utilised for the synthesis of selenoprotein. Selenide is highly reactive and readily bound to proteins. The various types of selenoprotein along with their functions is presented in Table.1

Table 1. Selenoproteins, their location and possible functions (Beckett and Arthur, 2005)

Selenoprotein	Nomenclature	Principal location	Function
Cytosolic glutathione peroxidases (GPX)	GPX1	Tissue cytosol, red blood cells	Storage, antioxidant
Phospholipid hyperoxide GPX	GPX2	Intracellular membranes, particularly testes	Intracellular antioxidant
Plasma GPX	GPX3	Plasma, kidney, lung	Extra cellular antioxidant
Gastrointestinal GPX	GPX4	Intestinal mucosa	Mucosal antioxidant
Epididymal GPX	GPX5	Epididymis	Weak antioxidant
Selenophosphate synthetase 2	SPS-2	Ubiquitous	SeCys biosynthesis
Iodothyronine 5^1-deiodinase type I	ID1	Liver, kidney, muscle	Conversion of T_4 to T_3
Iodothyronine 5^1-deiodinase type II	ID2	Placenta	Conversion of T_4 to T_3
Iodothyronine 5^1-deiodinase type III	ID3	Placenta	Conversion of T_4 to T_3
Thioredoxin reductase 1 and 2	TR1 and 2	Kidney, brain	Redox cycling
Selenoprotein N	SePN	Muscle	Cell proliferation
Selenoprotein P	SePP	Plasma	Transport, metal detoxifier
Selenoprotein R	SePR	Liver, kidney	Methionine sulfoxide reductase
Selenoprotein W	SePW	Muscle	Antioxidant, calcium-binding

DIFFERENCES BETWEEN ORGANIC SELENIUM (SE-YEAST) AND INORGANIC SELENIUM

Organic Se compounds can differ substantially, depending on the plant material analysed and a range of selenocompounds have been detected. Analytical speciation studies showed that the bulk of the Se in Se garlic and Se-yeast are in the form of gamma-glutamyl-Semethyl-selenocysteine (73%) and SeMet (85%), respectively (Ip et al., 2000) [Fig.1 and Fig.2].

Se-methylselenocysteine is the major selenocompound in Se-enriched plants such as garlic, onions, broccoli florets and sprouts, and wild leeks (Whanger, 2002). Major differences between organic selenium (se-yeast) and inorganic selenium are presented in Table.2.

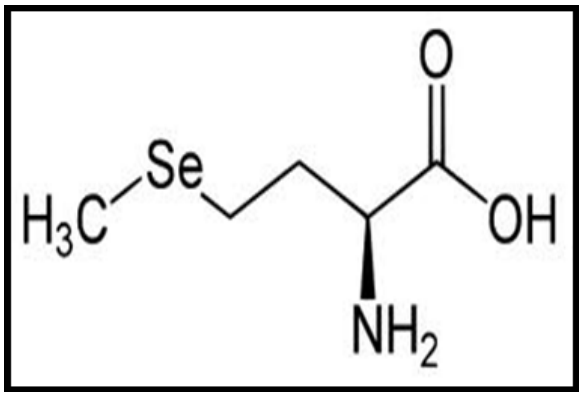

Fig.1. Selenomethionine

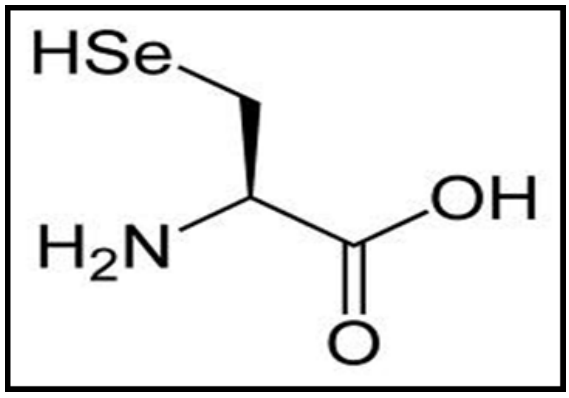

Fig.2.Selenocystine

Table 2. Major differences between organic selenium (Se-yeast) and selenite (Surai, 2006)

Attributes	Organic selenium	Selenite
Chemical forms	Selenomethionine, Selenocysteine	Sodium selenite, Sodium selenite, Calcium selenite
Biological functions	Increase maternal Se transfer to litter	Increase GSH-Px activity
Retention	High efficiency	Low efficiency
Excretion route	Urine	Faeces
Absorption	Similar to methionine with active transport in the gut	Similar to other minerals with passive transport in the gut
Accumulation	Building Se reserves by non-specific incorporation of SeMet into the proteins	Not accumulated in the body
Toxicity	At least 3 times less toxic than selenite	Highly toxic, can penetrate via skin causing problems
Bioavailability	Higher bioavailability in comparison to selenite to animals and humans	Very low availability for ruminants due to reduction by rumen microbes
Antioxidant activity	SeMet possess antioxidant properties and could scavenge no of free radicals	Possesses pro-oxidant properties and could stimulate free radical production when reacting with GSH

PREPARATION OF SELENIUM YEAST

Yeast (*Saccharomyces cerevisiae*) was reported as early as 1961 to take up inorganic Se from the culture medium and to convert it into selenomethionine (Blau, 1961). The biosynthesis of selenomethionine is known to occur in analogy to that of methionine (Schrauzer, 2003). Se yeast is manufactured by slowly adding sodium selenite to yeast culture during growth of the organism. In this case, the yeast's metabolism reduces selenite to selenide and incorporates it into cellular constituents in place of sulphur. The maximum amount of Se, a yeast cell can theoretically incorporate depends on its methionine content. However, the full replacement of methionine by selenomethionine is not possible. Selenomethionine is the major species identified in the proteolytic extract, accounting for approximately 60-84% of total Se species in the Se enriched yeast product (Rayman, 2002).

FUNCTIONS OF ORGANIC SELENIUM

Organic Se plays a crucial and ubiquitous role in the organism. The health benefits of Se supplementation in ruminants are well recognized. These are as follows:

Enhanced animal performance and growth

Significant growth improvement with organic Se supplementation was observed in animals. Shi et al. (2011) observed increased body weight gain in goats given with 0.3 ppm Se as Se yeast for 90 days as compared to control. Zavodnik et al. (2011) also observed 7.1% higher body weight gain in pigs fed with concentrate mixture containing 250 g Se yeast per ton for 178 days as compared to control animals.

Enhanced immune functions and immune defences

Injection of selenium (1mg Se kg^{-1} body weight) either alone or in combination with vitamin E (8 IU kg^{-1} body weight) significantly improved the production of specific antibodies against *E. coli* in cows (Panousis et al., 2001). Kumar et al. (2009) observed increased humoral immunity in lambs given 0.15 ppm of organic selenium (Jevsel-101) for 90 days than inorganic selenium supplemented animals. However, Dominguez-vara et al. (2009) observed that feeding of 0.3ppm organic Se to Rambouillet sheep for 95 days had no effect on plasma IgG concentration.

Prevent oxidative stress in animals

Studies on chicken (Payne and Southern, 2005) and pigs (Mahan and Parret, 1996) have shown that organic Se is deposited more effectively in muscle than inorganic Se. This increased tissue concentration of Se not only decrease oxidative stress but also protect unsaturated fatty acids from peroxidation damage (Korniluk et al., 2007). Kaur et al. (2003) observed higher activity of antioxidant enzymes (lipid peroxidase, catalase and glutathione peroxidase) by supplementation of organic Se for 6 weeks.

Enhanced reproductive functions

Selenium-yeast is essential for improving reproductive performance in animals (Surai, 2002). Marin-Guzman et al. (1997) reported that boars supplemented with selenium-yeast had a better sperm quality than the control non supplemented boars. The sperm motility (87.9% vs. 60.4%) and normal sperm (61.9% vs. 24.2%) count were significantly higher in supplemented compare to non supplemented animals.

Improved meat quality

Se yeast reduced meat drip loss and the incidence of pale soft, exudative meat (Downs et al., 2000). It also improved the shelf life during refrigeration (Skrivan et al., 2008). Zavodnik et al. (2011) observed 32% reduction in TBARS (thiobarbituric acid reactive substance) concentration and 0.92% increase in water holding capacity of pork obtained from pigs fed with concentrate mixture containing 250 g selenium yeast per ton for 178 days.

ORGANIC SELENIUM FOR RUMINANTS

Se plays very important role in ruminant nutrition. In many places in the world the Se levels

in feed ingredients are not adequate to meet the high Se demand of growing, reproducing and lactating animals. The common practise of dietary Se supplementation in an inorganic form has proved to be of low efficiency (Surai, 2006). The replacement of sodium selenite by organic Se sources, in particular, by selenized yeast in the form of Sel-Plex, has been proven to be an effective means of solving Se problems in the dairy, beef and sheep industries. Supplementation of organic Se causes an increased in Se concentration in blood, colostrum, milk and higher Se transfer via placental. Importance of organic Se in ruminants has been presented in Table 3.

Table 3. Importance of organic selenium for ruminants (Surai, 2006)

Parameter	Effect of organic vs. inorganic selenium	References
Growth rate	Increased	Bobcek et al., 2004
FCR	Improved	Bobcek et al., 2004
Somatic cell counts	Decreased	Eliott et al., 2005.
Retained placenta	Reduced	Erokhin and Nikonov, 2001; Eliott et al., 2005
Post partum endometritis morbidity	Decreased	Erokhin and Nikonov, 2001; Eliott et al., 2005
Services per conception	Decreased	Erokhin and Nikonov, 2001
Drip loss	Decreased	Simek et al., 2002
Se in cow plasma	Increased	Pehrson et al., 1999
Se in cow milk	Increased	Malbe et al., 1995
GSH-Px in erythrocytes	Increased	Malbe et al., 1995
Triiodothyronine (T_3) in plasma	Increased	Awadeh et al., 1998
Se in skeletal muscles	Increased	Simek et al., 2002

ORGANIC SELENIUM FOR POULTRY

Se is a choice for diets designed to maintain a high productive and reproductive performance of poultry. Replacement of sodium selenite by organic Se in the form of Se-Yeast (Sel- Plex) in the breeder diet is related to an improvement of fertility, hatchability and viability of chicks in early postnatal development (Surai, 2006). Indeed, organic Se is more effectively transferred from the diet to the egg and further to the developing embryo. This improves antioxidant defences and helps chickens overcome the oxidative stress of hatching, leading to improvement of hatchability (Surai, 2006). Data indicate that Se transferred from the egg to the embryo as a result of organic Se supplementation of the maternal diet had positive effect on the Se status of the developing chicks up to 4 weeks post hatch (Pappas et al., 2005). It is well recognised that egg shell consists of about 95% of minerals and 5% organic matrix. This 5% of the organic matrix determines shell quality. Since organic Se is an integral part of the organic matrix it was suggested that it could affect shell quality.

ORGANIC SELENIUM FOR PIGS

Increased Se transfer via placenta, colostrum and milk through organic Se would improve the antioxidant defences of the piglets and would be beneficial for the piglet's general health. It is well established that a low-Se maternal diet is a risk factor for the sow and the developing pig embryo. Importance of organic Se in ruminants has been presented in Table 4.

Table 4. Importance of organic selenium for pigs (Surai, 2006)

Parameter	Effect of organic vs. inorganic selenium	References
Growth rate	Increased	Janyk, 2001
FCR	Improved	Bobcek et al., 2004
Drip loss	Decreased	Mahan et al., 1999
Tissue Se concentration	Increased	Mahan et al., 1999
Meat colour	Improved	Mahan et al., 1999
Liver Se	Increased	Ortman and Pehrson, 1998
Blood Se	Increased	Ortman and Pehrson, 1998
Placental Se transfer	Increased	Mahan and Kim, 1996
Muscle Se level	Increased	Mahan and Parrett, 1996
Loin-eye area	Increased	Miller et al., 1997.
Se excretion	Decreased	Mahan and Parrett, 1996
Back fat depths	Decreased	Wolter et al., 1999
Se bioavailability in sow milk to the nursing pig	Increased	Mahan and Parrett, 1996
Piglet weight at birth and weaning and daily gain	Increased	Janyk, 2001
Total piglet born and piglet born alive	Increased	Pineda et al., 2004
Pre-weaning mortality	Reduced	Lampe et al., 2005

SELENIUM DEFICIENCY

Poultry

Se was originally considered as a toxic element, but in 1957 Schwarz and Foltz recognized Se to be the effective component of "factor 3" which prevented liver necrosis in rats. Schwarz and Foltz (1957) further demonstrated that Se prevented exudative diathesis in chicks. Se deficiency in chicks caused reduced egg production and hatchability in poultry (Cantor and Scott, 1974); poor growth, increased mortality and gizzard myopathy in young turkey poults (Scott et al., 1967). Pancreatic fibrosis also occurred in severe Se deficient chicks (Noguch et al., 1973). Chicks fed the Se-free diet showed severe degeneration and fibrosis of the pancreas (Thomson and Scott, 1970).

Ruminants

Se deficiency is related to several nutritional disease conditions in animal. Deficiencies of Se in cattle and sheep have been observed under natural grazing conditions in many countries of the world. Signs of Se inadequacy such as white muscle disease (nutritional muscular dystrophy) occur primarily in young calves or lambs when born to Se deficient dams. Infertility has increased in ewes grazing pastures low in Se (Schwarz, 1976). The diseases associated with Se deficiency are White muscle disease in new born lamb, Weiner ill thrift (or selenium-responsive ill-thrift) and Scouring in young sheep *etc*.

SELENIUM TOXICITY

Poultry

Se toxicity leads to poor hatchability of chicken eggs (Franke, 1934). Embryos were found

with many types of deformities. Legs, toes, wings, beaks, and eyes were often malformed, rudimentary, or entirely lacking (Franke and Tully, 1935).

Ruminant

Steers fed with 10 and 25 ppm, as selenomethionine or sodium selenite respectively suffered alkali disease and blind stagger (O'Toole and Raisbeck, 1995). Consequently, organic Se is more toxic in ruminant compared with inorganic Se. The distinctive histological changes that developed in the hooves, particularly in stratum medium, may account for the dystrophic digital lesions in selenosis.

Swine

Acute Se toxicities in young pigs can result in clinical selenosis disease symptoms similar to those for lambs and calves (Shortridge et al., 1971). Chronic Se poisoning in pigs is recognized by dullness, lack of vitality, emaciation, roughness of hair coat, loss of hair, soreness and sloughing of hooves, stiffness and lameness due to erosion of the joints of long bones, atrophy of the heart, cirrhosis of the liver and anemia (Underwood, 1977). Acute oral intoxication in swine resulted in vomiting, diarrhea, paresis, anorexia, trembling, and depression (Miller and Williams, 1940).

SELENIUM-ENRICHED EGGS, MEAT AND MILK

Since the Se content in plant-based food depends on its availability from soil, the level of this element in human foods varies among regions. In general eggs and meat are considered to be good sources of Se in the human diet. When considering ways to improve human Se intake, there are several potential options. These are direct supplementation, soil fertilisation, supplementation of food staples such as flour and production of Se-enriched functional foods *etc.* Some examples of se-enriched eggs produced in various countries have been presented in Table 5.

Table 5. Some examples of se-enriched eggs produced in various countries (Surai, 2006)

Trade name	Countries
NutriPlus / LTK omega plus / Selenium plus	Malaysia
Selen egg/ Doctor hen egg	Thailand
Organic selenium egg	Singapore
Mr egg	Mexico
Heart beat eggs	New Zealand
Tavasyumurta/ Sekeryumurta/ Selenium eggs	Turkey
SelPlex eggs	Switzerland
NutriPlus	Portugal
Omega pluss	Hungary
Bag of life (Koshikzhitja)/ Spring of life (Dzherelozhitja)	Ukraine
Rejuvenating (Molodiljnije) /Aksais' sun (Aksaiskoyesolnishko) / Spring of cheerfulness (Rodnikbodrosti)	Russia
Mettlesome eggs (Molodetskoye)	Belarus
Columbus	UK, Belgium, Netherlands, France, Spain, USA, Japan, South Africa, India, Israel, Korea, Australia

CONCLUSION

The analysis of the literature presented above, reinforces the importance of organic Se in animal and human nutrition and health. Indeed, the global Se inadequacy is responsible for an increased susceptibility to various diseases, including major modern killers such as cancer and cardio-vascular diseases. Optimisation of Se nutrition of poultry and farm animals will result in increased efficiency of egg, meat and milk production and even more important, will improve quality. From the data presented above it is clear that the main lesson which we have to learn from nature is how to use organic Se in animal and human diets.

ACKNOWLEDGEMENT

I sincerely acknowledge and thank all the researchers for their valuable contributions included in this pursuit.

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e-planet **14** (2): 20-27 (December 2016)

Composition of natural food of long whiskers catfish, *Mystus gulio* (Hamilton, 1822) from Chilika lake

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Date of receipt: 10. 10. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

Study was undertaken to analyze the natural food composition of *Mystus gulio*, a catfish having high commercial value in Chilika lake. In total 359 fresh fish specimens were collected during March-December, 2012 from different regions of the lake. The qualitative and quantitative analyses of stomach contents of the collected specimens were done by percentage of frequency occurrence method. The obtained results exhibited that *M. gulio* fed on variety of food items such as amphipods (44.11%), copepods (11.88%), insects (9.24%), aquatic plants (7.96%), prawns (4.38%), fish (4.38%), diatoms (1.49%), mysis (1.38%), cladocerans (1.16%), rotifers (0.65%), isopods (0.63%) and gastropods (0.39%). Among these, amphipods appeared as one of the most dominant groups, commonly observed in the macrophyte dominated areas. Most of these groups were recognized under macro-zoobenthic groups. The present study indicated that the species preferred mostly on animal food attached on the plant materials. So, the fish might be categorized as omnivore species having mixed food of both animal and plant origin.

Key words: *Mystus gulio*, natural food, Chilika lake, brackish water

INTRODUCTION

Food is one of the important criterion for any of the fish species from management perspective particularly in the shallow coastal ecosystem like Chilika lake. It is the main source of energy and plays an important role in determining the population levels, rate of growth and condition of fish. Thus, food and feeding habits of fish play a significant role in aquaculture practice as well as for better management of fisheries resources. Such information helps to select those species of fishes for culture which will utilize all the available potential food of the targeted water bodies without any competition with one another but, will live in association with other fishes (Begum et al., 2008). Feeding is the dominant activity of the entire life cycle of fish (Royce, 1972; Gupta and Banerjee, 2014). The success of planning and management of fishery depends on the knowledge of their biology in which food and feeding habits include a valuable portion. The occurrence, distribution and abundance of fish stock mainly depend on the availability of food. Hence, the quantum of fish production is directly related with the nutrition available to it (Arthi et al., 2011). Therefore, study of the natural foods and its composition in fish species is a subject of continuous research because it continues the basis for the

development of a successful fisheries management (Oronsaye and Nakpodia, 2005). Analysis of contents in the gut and features of the alimentary system can provide maximum relevant information on foods, its composition and selectivity, if any. In addition, other information such as feeding source and relevant area of foods can also be revealed, which may be an indicator of immediate risks of direct predation upon themselves (Paszkowski et al., 1996). Furthermore, in-depth analysis of all food-items of fish can be able to predict possible risk of bioaccumulation in the body through its food compositions.

Mystus gulio (Siluriformes: Bagridae), the long whiskers catfish well known as *Kantia* (in Odia), is primarily a brackish water fish that enters and lives in fresh water bodies like rivers and streams. This catfish is well distributed throughout Chilika having high commercial importance (Mohanty et al., 2015). For the conservation and management point of view, the species in "Lake Ecosystem" needs a holistic research about the biology of the fish species.

MATERIALS AND METHODS

Chilika, the largest brackish water lake of Asia lies in the east coast of India, situated between latitudes 19°28'-19°54' North and longitude 85°05'-85°38' East. Chilika is designated as an important Ramsar site (No. 229) of India on October, 1981. The water spread area of the lake varies between 906 km^2 to 1165 km^2 during summer and monsoon respectively. The estuarine lake is a unique assemblage of marine, brackish and fresh water eco-systems.

The lake is divided into four ecological sectors namely; the southern sector, the central sector, the northern sector and the outer channel sector (Fig.1). Basically, the northern sector is fresh water dominated zone, central sector is a brackish water zone, southern sector is a higher saline area and the outer channel sector is marine in nature with saline water but during monsoon, water becomes fresh due to discharge of flood water of the lake to the sea through this area (Fig. 1).

A total of 359 fresh fish specimens were collected from Balugaon and Kalupadaghat fish landing centre of Chilika during March and April (for pre-monsoon season), July and August (for monsoon season), November and December (for post-monsoon season) of 2012. Fresh fish specimens were collected from fishing boats, immediately after arrival in the landing centers. Then, the fishes were captured by using gill nets (mesh 26-42 mm) and screen barrier net / fixed net (mesh 14-22 mm).

After collection, the specimens were transported to research laboratory in polythene bags. The specimens were selected randomly in spite of their size, maturity and sex groups.

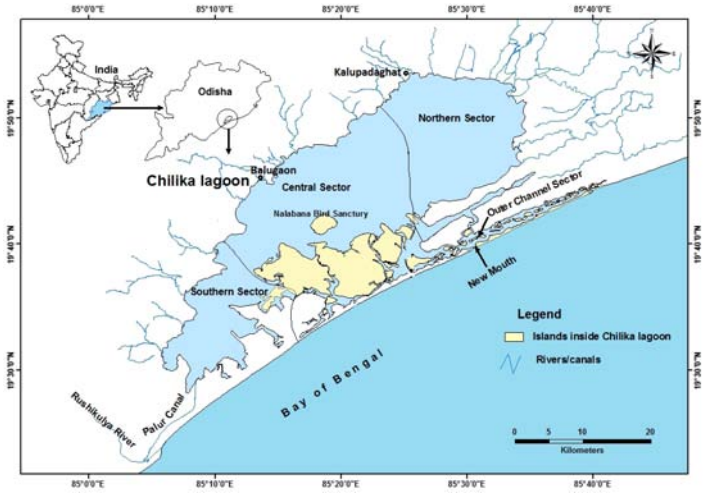

Fig. 1. Location map of Chilika lake indicating sampling sites

In the laboratory, specimens were washed and cleaned properly with tap water. The total length (TL in mm), body weight (BW in g) of each specimen was recorded and then dissected by scissors to remove the guts. The sexes were identified by gonads. Each gut was preserved in separate container with 7% formalin solution to prevent the breakdown of the food materials. The bowels were properly labeled with respective length, weight, sex, date and place of collection.

RESULTS AND DISCUSSION

During analysis, the entire gut was cut-open based on different length group and all the food items were collected into a petri-dish with the help of a fine forceps. The food items were examined and identified by following the keys of Pennak (1953), Ward and Whipple (1959), Needham and Needham (1962) in a dissecting microscope. The stomachs were graded by observing their fullness.

To apply points to each food items, all the contents were first identified. The points were assigned to each food items depending on their number and volume. Then the points recorded by different food items were summed-up and their percentages were calculated from the total number of points gathered by all food items.

$$\text{Points of each food item (\%)} = \frac{\text{No of points assigned to the particular food item}}{\text{Sum of all points assigned to all the food items}} \text{ X } 100$$

Frequency of occurrence is the number of times a particular food item occurred in the gut is counted and expressed as a percentage of the total number of stomachs with foods (Ikongbeh et al., 2014). Here, the empty stomachs were not included.

$$\text{Occurrence (\%)} = \frac{\text{Total no.of stomach with the particular food item}}{\text{Total no.of stomachs with food}} \text{ X } 100$$

Feeding Index, a measure of level of fullness of the stomachs were recorded irrespective of the occupancy of the stomachs by food items using '0' for empty; '1' for less, '2' for half, '3' for more than half and '4' for full stomach.

The details of each food items estimated from the stomach content analysis of *Mystus gulio* is presented in Fig. 2. The overall analysis showed that the fish feeds on variety of food items. The constituents were categorized into 13 groups. Considering the volume of individual groups of food items according to the assigned points, amphipods accounted for 44.11% predominated as a single item among the composition.

Copepods accounted for 11.88%, crustacean parts 9.6% and insects 9.24% ranked 2nd, 3rd and 4th respectively. The other important items were aquatic plants 7.96%, prawns 4.38%, fish derivatives 4.38%, diatoms 1.49 %, mysis 1.38%, cladocerans 1.16%, rotifers 0.65%, isopods 0.63%, and gastropods 0.39%

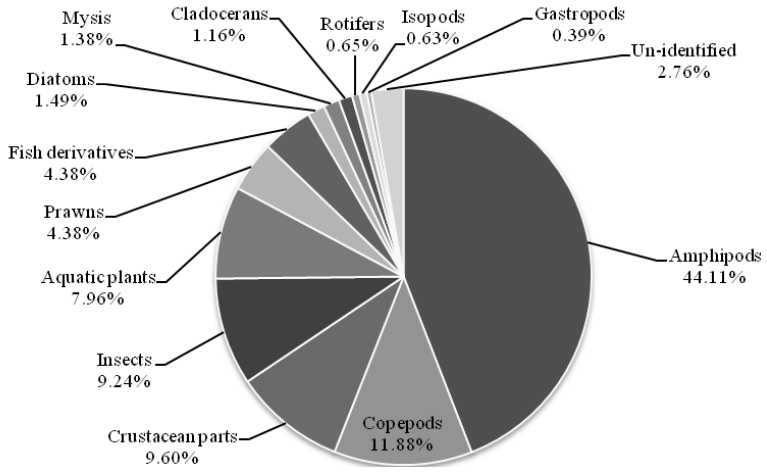

Fig. 2. Percentage of food composition of *Mystus gulio* from Chilika

The composition (in %) of food items were analyzed seasonally, presented in Table 1.

Table 1. Seasonal food composition (in %) of *Mystus gulio* during pre-monsoon, monsoon and post-monsoon period from Chilika.

Food items	Pre-monsoon		Monsoon		Post-monsoon		All season	
	Total points (%)	Frequency occurrence (%)	Total points (%)	Frequency occurrence (%)	Total points (%)	Frequency occurrence (%)	Total points (%)	Frequency occurrence (%)
Amphipods	46.77	80.77	41.55	85.82	45.72	85.71	44.11	84.94
Copepods	13.56	57.69	13.95	51.49	9.00	38.89	11.88	47.44
Crustaceans	9.62	21.15	8.40	20.15	10.88	27.78	9.6	23.4
Insects	5.58	28.85	9.23	29.1	10.76	38.1	9.24	32.69
Aquatic plants	5.37	30.77	9.28	31.34	7.62	28.57	7.96	30.13
Prawns	9.08	17.31	4.89	10.45	1.89	10.32	4.38	11.54
Fish derivatives	6.54	15.38	3.38	8.21	4.56	16.67	4.38	12.82
Diatoms	0.69	11.54	0.63	5.97	2.72	16.67	1.49	11.22
Mysis	0.52	5.77	1.53	11.94	1.56	12.7	1.38	11.22
Cladocerans	0.00	0	2.15	17.16	0.60	5.56	1.16	15.06
Rotifers	0.58	7.69	0.74	11.19	0.58	6.35	0.65	8.65
Isopods	0.00	0	0.93	10.45	0.56	9.52	0.63	8.33
Gastropods	0.00	0	0.40	6.72	0.56	7.14	0.39	5.77
Un-identified	1.71	21.15	2.95	32.09	3.00	31.75	2.76	30.13

Amphipods formed the principal diet irrespective of season. In pre-monsoon, amphipods alone contributed 46.77% of all food items followed by copepods 13.56%, crustacean parts 9.62%, prawns 9.08%, fish derivatives 6.54%, insects 5.58 % and aquatic plants 5.37%. Other food items i.e., diatoms, mysis, cladocerans and rotifers were found of very negligible amount. No isopods or gastropods were recorded during the season.

During monsoon, amphipods were found as most preferred food items with 41.55% followed by copepods 13.95%, aquatic plants 9.28%, insects 9.23%, crustacean parts 8.4%, prawns 4.89%, fish derivatives 3.38%, cladocerans 2.15% and mysis 1.53%. Similarly, in post-monsoon, amphipods registered 45.72% followed by crustacean parts 10.88%, insects 10.76%, copepods 9%, aquatic plants 7.62%, fish derivatives 4.56%, diatoms 2.72 %, prawns 1.89% and mysis 1.56% as shown in Table 1.

Composition of individual food items encountered from fish guts during pre-monsoon, monsoon and post-monsoon season are estimated those are presented in details in Fig. 3. The results indicated that major food items were found in the guts of the species in all the three seasons. All other minor items also found in almost all the seasons except that of cladocerans, isopods and gastropods; those were absent in the fish gut during pre-monsoon season.

A visible distinction is found in the food composition of *Mystus gulio* within different size groups (Table 2).

If we analyze Table 2, the preferences of amphipods were found to be most dominant, among all sized groups, but its magnitude decreased with increase in fish size (TL – total length). Amphipods were found about 2/3rd of all items i.e., 63.58% in smaller sized (TL > 100 mm) individuals but 45% in medium sized groups (TL 100 - 150 mm) and only 34.24% in larger sized individuals (TL 150 mm <). Other items like prawns, detritus, fishes and insects were found in more quantity in the guts of bigger sized fish (TL 150 mm <). The proportion

of all these items found decreased with decrease in fish size. No fish, prawns and gastropods were found in the guts of smaller sized (TL > 100 mm) individuals. Isopods, mysis, cladocerans, rotifers were found mostly in smaller individuals than larger sized fishes. No such big differences were identified in preferred food items with sex differences (Table 2).

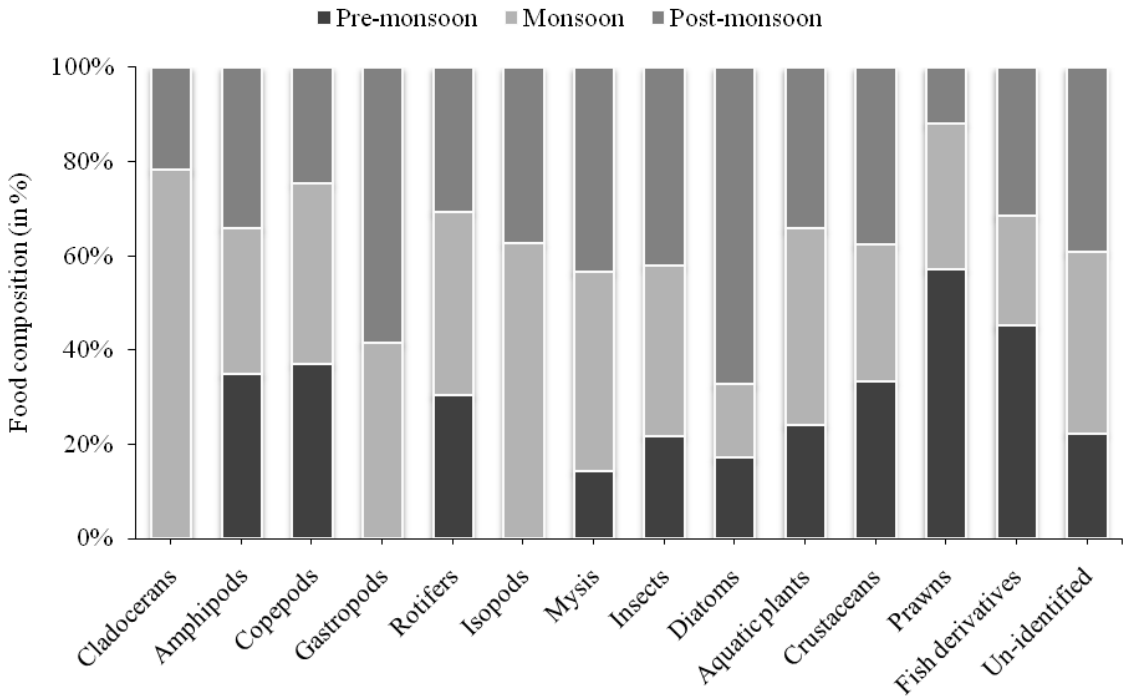

Fig. 3. Seasonal variation of composition (in %) of individual food items of *Mystus gulio*

Table 2. Composition of food items (% points) in relation to fish size and sex of *Mystus gulio*

Food items	TL >100mm	100-150mm TL	150mm< TL	Male	Female
Amphipods	63.58	46.43	34.24	45.23	43.34
Copepods	9.75	12.19	10.29	10.87	12.58
Crustaceans	1.67	8.71	13.39	10.07	9.28
Insects	2.75	8.34	12.20	8.57	9.70
Aquatic plants	4.75	8.78	2.84	9.17	7.12
Prawns	0.00	4.26	5.57	3.98	4.65
Fish derivatives	0.00	2.88	12.14	3.31	5.12
Diatoms	2.50	1.47	1.76	2.02	1.12
Mysis	7.50	1.32	1.84	0.79	1.78
Cladocerans	2.50	1.39	0.20	1.64	0.84
Rotifers	2.50	0.63	0.75	0.57	0.70
Isopods	0.83	0.52	1.06	0.91	0.43
Gastropods	0.00	0.36	0.61	0.54	0.29
Un-identified	1.67	2.73	3.12	2.35	3.04

The frequency of occurrences (in %) of each food items showed the presence of amphipods were found more frequently in the guts than other items. Amphipods accounted for about 85% (i.e., 85% of all guts had amphipods excluding the empty guts). Similarly, copepods found in 47.44% followed by insects 32.69%, plant matters 30.13%, crustacean parts 23.4%, cladocerans 15.06%, fish derivatives 12.82%, prawns 11.54 %, mysis 11.22%, diatoms 11.22%, rotifers 8.65%, isopods 8.33% and gastropods 5.77%. The seasonal variation of frequency of occurrences (in %) is also described for each food items separately in Table 1.

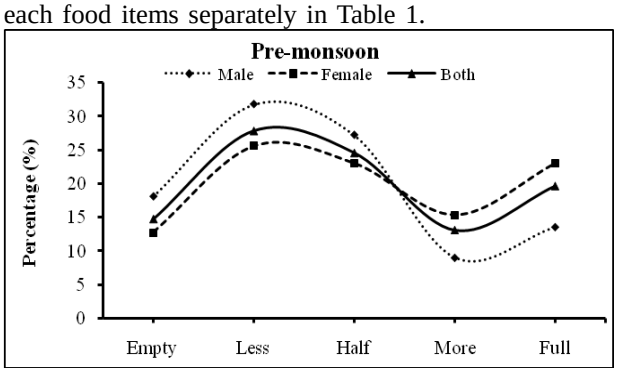

Fig. 4. The stomach fullness status of *Mystus gulio* during pre-monsoon season

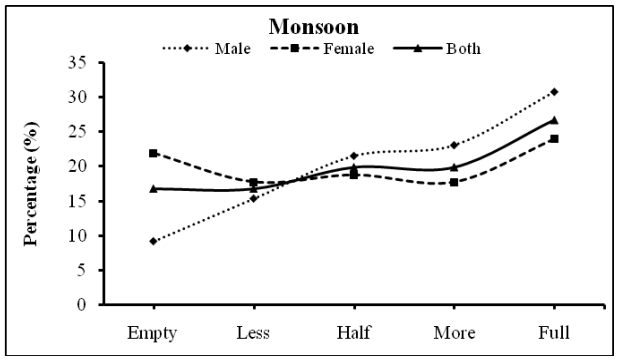

Fig. 5. The stomach fullness status of *Mystus gulio* during monsoon season

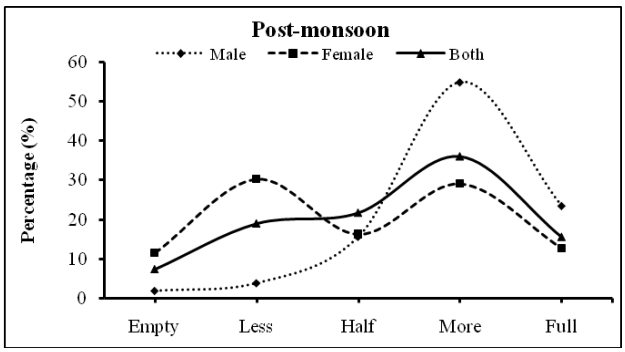

Fig. 6. The stomach fullness status of *Mystus gulio* during post-monsoon season

The stomach fullness status (feeding index) of individual specimen was analyzed in relation to sex and seasons, presented in Fig. 4-6. In pre-monsoon, the stomach fullness status of 'less' and 'half' were dominant in both the sexes (Fig. 4) but in monsoon, the stomach status of all the categories were found in equal proportion in females and in males a gradual increase in specimens number was found from status 'empty' to 'full' (Fig. 5). In post-monsoon, there were very few male specimens found with stomach status 'empty' or 'less' (Fig. 6). But in females, the fluctuated status was recognized. So, in overall seasonal stomach status was concerned, in pre-monsoon the stomachs found with medium food and in monsoon and post-monsoon, the status was found more than half to full.

Present study clearly characterized that the food of *Mystus gulio* was consisting of amphipods, copepods, insects, aquatic plants, prawns, fish, diatoms, mysis, cladocerans, rotifers, isopods, gastropods and other crustaceans. Among these, amphipods were found predominant in stomachs of all the fish categories i.e., in seasons (pre-monsoon, monsoon and post-monsoon), sexes (male and female) and sizes (TL > 100 mm, 100 - 150 mm and 150 mm <). Similar findings were also reported by Jhingran and Natarajan (1966) from Chilika and ranked amphipods as most dominant food items followed by prawns. Although the present study differs little in percentage composition but the constituents of overall food items mentioned by Jhingran and Natarajan (1966) seems to be similar.

The present finding of plant matters (including algae), fish, mysis, gastropods, isopods, insects and copepods were the main food items of *M. gulio* from the lake which corroborates with the findings of Jhingran and Natarajan (1966). Similar findings also reported from many water bodies like Hooghly River (Pantulu, 1961) and Minakhan wetlands (Yusuf and Majumdar, 1993). Pantulu (1961) mentioned that prawns, fish, insects, copepods and cladocerans are the preferred food of *Mystus gulio* from Hooghly River whereas, Yusuf and Majumdar (1993) categorized the fish *Mystus gulio* as a bottom feeder, feeds mostly on zooplanktons like copepods, cladocerans and rotifers.

Seasonal variation in composition (in %) of food items is concerned, no much variations was identified in the contribution of amphipods (Mean±SD; 44.11±2.76) in the stomachs, confirmed the most preferred items, particularly in Chilika ecosystem which was also confirmed by Jhingran and Natarajan (1966). However, in seasonal variation of prawns in the stomachs showed its high presence during pre-monsoon season than other season, might be the reason that the prawn abundance during this season (February-April) is very high (Bhatta and Panda, 2008). So that the fish *M. gulio* can able to assemble more prawns (of small sized) easily as their prey.

As the fish grew in size, there appeared to be the rapid changes in food preferences. With increase in body size, the food preferences changed from smaller plankton, benthos to larger soft and hardy shelled individuals like prawns, insects, other crustaceans, fish etc. This is strongly supported from the results as found in the present study, that no fish or prawns were noticed from the stomachs of fish sized TL > 100 mm, but it appeared very less amount in medium sized fish (TL 100-150 mm) and the sized more than 150 mm (TL) voraciously feeds on fish and prawns. But no such noticeable differences were found among males and females.

The stomach fullness condition of fish is associated with their breeding conditions. According to Jhingran and Natarajan (1966), *Mystus gulio* breeds in Chilika during June to November and another in January. The results of the present study of the seasonal feeding status, it is observed that less to medium foods were found in the stomachs during pre-monsoon. This was due to reduction in the feeding intensity by the berried fish before spawning. These findings support the study undertaken by Begum et al. (2008).

The feeding rate gradually increased in monsoon and highest in post-monsoon seasons. This indicates, the fish feeds voraciously in later stage of post-monsoon when the fish spawn mature enough to overcome from the stress during spawning. Similar conclusions also observed from the studies of various workers like Pandian (1966), Homans and Vladykov (1954) reported that an inverse relationship between feeding and breeding cycles. Pantulu (1966) also reported a similar observation from his studies on the feeding intensity of *M. gulio* in the Hooghly estuary of India. It was mentioned that, more 'empty' stomachs were noticed during the pre-spawning but after spawning in July, the fish fed intensively with either full or 'gorged' stomach. This high feeding intensity steadily decreased during the subsequent months (during December-January). In the pre-monsoon period, the stomachs occupied with less to medium quantity of food. These findings support the findings of Pandian (1966). According to him, the stomachs of the fishes collected in summer months revealed that more than 80% were starving and the rest had only tracer quantities of food in the stomachs. Many world reports categorized the fish as omnivore species having mixed diet of both animal and plant origin (Yusuf and Majumdar, 1993; Rajkumar et al., 2013). *Mystus gulio* feeds predominantly on food of animal origin but also consume plants (Yusuf and Majumdar, 1993). Similarly, Begum et al., (2008) suggested after an extensive study on the food items of *Mystus gulio* that they are euryphagous (*i.e.* feeding on a wide range of organisms). Such a euryphagous feeding behavior is documented in most of the species of catfishes (Thomas, 1966).

CONLCUSION

It is important to decipher that the species prefers most on animal food than plants in the Chilika lake. It was also observed that *M. gulio* can be classified as an omnivorous feeder as the diet covers a wide spectrum of food ranging from various types of plankton to benthic invertebrates and plants. The fish also exhibits an overlapping in food and feeding habits in order to avoid inter and intra specific competition for available food.

ACKNOWLEDGEMENT

The authors are thankful to the Professor and HOD, Department of Zoology, Utkal University, Bhubaneswar, Odisha (India) to extend the facilities for the above study.

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e-planet **14** (2): 28-32 (December 2016)

Conjunctive effect of graded levels of fly ash and recommended dose of fertilizer on yield, nutrient uptake and quality of sunflower grown on Vertisol

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Date of receipt: 18. 09. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

To study the effect of graded levels on yield, uptake of nutrients and quality of sunflower (var. EC 68415), a field investigation was conducted during 2005-06 with seven levels of fly ash (0, 20, 40, 60, 80, 100 and 120 t ha^{-1}) along with recommended dose of NPK (60:60:00) in Vertisol. The results revealed that application of 40 t ha^{-1}, 60 t ha^{-1} of fly ash in combination with recommended dose of NPK (60:60:00) were *at par* with each other and significantly superior over RDF and rest of the treatments. Lower levels of fly ash application @ 60 t ha^{-1} increased in seed (6.54 q ha^{-1}) and straw (12.51 q ha^{-1}) yield as against 5.66 q ha^{-1} and 11.80 q ha^{-1} respectively in RDF. Results showed that significantly highest content, uptake of nutrients and quality of sunflower were recorded with the increasing level of fly ash up to 60 t ha^{-1}.

Key words: Fly ash, sunflower yield, nutrient uptake, Vertisol

INTRODUCTION

Coal is burnt to generate electricity at thermal power station. Coal upon its complete burning yields about 30-40% of fly ash by weight and about 1.35 M^3 space is required to dump one ton of fly ash. The disposal of fly-ash thus generated is a perpetual problem. It is expected that use of fly-ash instead of lime in agriculture can reduce net CO_2 emission and also reduce global warming (Kishor et al., 2010). Plants grown on sulphur deficient soil respond favorably to incorporation of fly ash in to the soil. Warambhe et al. (1992) reported that increased N, P and K content in plant by the application of fly-ash. Arivazhagan et al. (2011) concluded from NTPC-Vindhyachal field trials that the application of fly ash @ 50 t ha^{-1} increased the yield of maize crop from 36-40%, red gram from 55-58%, mustard from 28-32 % and potato from 25-37 % over control.

Therefore, the present study on utilization of flyash was undertaken to assess effect of flyash application at recommended dose of NPK on yield, nutrient uptake as well as quality of sunflower in black cotton soil.

MATERIALS AND METHODS

The experiment was conducted at Central Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) on sunflower (var. EC

68415), as a test crop. The fly ash was brought from thermal power station, Paras, Tal. Balapur, Distt. Akola situated at 22° 42' N and 77° 02' E at 307.42 m above mean sea level).

The field experiment was carried out during 2005-06 with seven treatments of graded levels of fly ash and recommended dose of NPK (60:60:00) [Table-1]. The experiment was laid out in RBD with four replications.

Table 1. Field experiment during 2005-06 with seven treatments

Treatments	Details
T_1	RDF (No fly ash)
T_2	20 t ha^{-1} Fly ash + RDF
T_3	40 t ha^{-1} Fly ash + RDF
T_4	60 t ha^{-1} Fly ash + RDF
T_5	80 t ha^{-1} Fly ash + RDF
T_6	100 t ha^{-1} Fly ash + RDF
T_7	120 t ha^{-1} Fly ash + RDF

The intercultural operations and plant protection measures were followed as and when required. The crop was harvested at maturity and grain and straw yield were recorded on plot basis and then converted on hectare basis. Treatment-wise plant samples were collected at harvest and seed samples after threshing were analysed for NPK content as per standard procedure suggested by Jackson (1978) and Piper (1966). The nutrient uptake was calculated by multiplying its per cent concentration with yield. Oil content was estimated by ether extraction method (Soxhlet extractor method) as outlined by Sankaram (1966) and statistical analysis as per Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

Effect of fly ash on seed and straw yield of sunflower

Application of fly ash at increasing level up to certain level in combination with recommended dose of NPK increased the grain and straw yield (Table 1). However application of 60 t ha^{-1} of fly ash and recommended dose of NPK recorded significantly higher yield of 6.54 and 12.51 q ha^{-1} seed and straw respectively as compared to RDF 5.66 and 11.80 q ha^{-1}. Similar results were observed by Kuchanwar et al. (1997), Kumar et al., (2005), Rizvi and Khan, (2009) and Arivazhagan et al. (2011).

Effect of fly ash on macronutrients uptake by sunflower

The data in Table 2, revealed that uptake of nitrogen and potassium in seed and straw was significant, however non significant results were noted in respect to uptake of phosphorus in sunflower. Application of fly ash at lower level (20, 40 and 60 t ha^{-1}) with recommended dose of NPK helped significantly higher uptake of nitrogen and potassium over RDF without fly ash.

The uptake of NPK in the treatments receiving fly ash at lower level (20, 40 and 60 t ha^{-1}) was more over RDF, however, further increase in doses of fly ash (80, 100 and 120 t ha^{-1}) tends to decrease uptake in seed and straw. The highest N, P and K uptake in seed and straw was recorded in treatment receiving 60 t ha^{-1} of fly ash and recommended dose of NPK which were 44.33, 3.40 and 7.19 kg ha^{-1} in seed and 17.57, 3.20 and 19.26 kg ha^{-1} in straw of sunflower respectively. The lowest value of NPK uptake was found in 120 t ha^{-1} fly ash treatment. Similar trend was observed by Bhaisare et al. (2000), Grewal et al. (2001), Thanunathan et al. (2001) and Basu et al., (2006).

Effect of fly ash on micronutrients content in seed and straw of sunflower

The data represented in Table 3 shows that different levels of fly ash with the combination of RDF recorded significant influence on the micro-nutrient content in seed as well as straw except in the manganese content in the seed of sunflower which was non-significant. The manganese content in the seed of sunflower was recorded non-significant influence due to various treatments. However, highest (0.093 ppm) concentration of Mn was observed in the treatment receiving flyash 60 t ha^{-1} and lowest 0.054 ppm in the RDF with no fly ash treatment. Highest Mn concentration in straw (0.19 ppm) was recorded with treatment receiving 60 t fly ash ha^{-1}.

Significant results of Zn concentration (0.29, 0.31, 0.34 ppm) in seed were observed in the treatments receiving 20, 40 and 60 t fly ash ha^{-1} over RDF (0.19 ppm). However, Zn content in straw was found significantly superior in the treatment 40 and 60 t fly ash ha^{-1} as compared to RDF (0.06 ppm). Application of 60 t ha^{-1} of fly ash and recommended dose of NPK recorded significantly highest copper concentration i.e. 0.15 ppm in seed as well as in straw as compared to RDF i.e. 0.09 ppm to 0.05 ppm in seed and straw respectively. This might be due to the element Zn is largely distributed on the surface of ash particles which remains unmeshed in the core of ash matrix (Theis and Wirth, 1997). Hence, the release of Zn might be resulting in its rapid increased concentration in sunflower.

Effect of fly ash on oil content in seed of sunflower

Sunflower being an oilseed crop its recovery of oil is an important parameter, hence oil percentage in seed was estimated and data presented in table 4. Application of fly ash at lower levels (20, 40 and 60 t ha^{-1}) showed increase in trend and higher level of fly ash (80, 100 and 120 t ha^{-1}) showed decrease in trend of oil content in seed of sunflower. Significantly highest oil content in seed of sunflower was recorded with 60 t ha^{-1} of fly ash and recommended dose of NPK (36.0%) as compared to RDF (30.4%). Significant increase in oil content may be due to the fact that fly ash contains sulphur, which is beneficial to oil seed crops.

CONCLUSION

Use of fly ash in soil should not be liberal, as study revealed that application of 60 t ha^{-1} of fly ash with recommended dose of NPK showed beneficial effect whereas, the higher doses of fly ash recorded negative influence on the yield, nutrient content, their uptake and quality of sunflower crop grown on Vertisol.

Table 2. Effect of fly ash on seed and straw yield of sunflower

Treatments	Seed Yield (q ha^{-1})	Straw Yield (q ha^{-1})
T_1 - RDF (No fly ash)	5.66	11.80
T_2 - 20 t ha^{-1} Fly ash + RDF	6.31	12.03
T_3 - 40 t ha^{-1} Fly ash + RDF	6.41	12.21
T_4 - 60 t ha^{-1} Fly ash + RDF	6.54	12.51
T_5 - 80 t ha^{-1} Fly ash + RDF	6.24	11.30
T_6 - 100 t ha^{-1} Fly ash + RDF	6.24	11.10
T_7 - 120 t ha^{-1} Fly ash + RDF	5.6	10.38
SE (m) $\pm$	0.075	0.21
CD at 5%	0.22	0.60

Table 3. Effect of fly ash on macronutrient uptake (kg ha^{-1}) in seed and straw of sunflower

Treatments	Nitrogen		Phosphorous		Potassium	
	Seed	Straw	Seed	Straw	Seed	Straw
T_1 - RDF (No fly ash)	35.05	15.33	3.0	2.8	5.03	16.52
T_2 - 20 t ha^{-1} Fly ash + RDF	40.08	15.57	2.9	2.9	6.18	17.80
T_3 - 40 t ha^{-1} Fly ash + RDF	40.29	17.26	3.2	2.7	6.53	18.31
T_4 - 60 t ha^{-1} Fly ash + RDF	44.33	17.57	3.4	3.2	7.19	19.26
T_5 - 80 t ha^{-1} Fly ash + RDF	37.35	15.56	3.0	2.7	5.42	16.61
T_6 - 100 t ha^{-1} Fly ash + RDF	33.79	14.73	2.9	2.6	5.24	15.42
T_7 - 120 t ha^{-1} Fly ash + RDF	33.07	14.35	3.0	2.5	4.42	14.22
SE (m) $\pm$	0.26	0.08	0.07	0.08	0.010	0.028
CD at 5%	0.73	0.24	0.20	0.23	0.030	0.080

Table 4. Effect of fly ash on micronutrient content (%) in seed and straw of sunflower

Treatments	Manganese		Zinc		Copper	
	Seed	Straw	Seed	Straw	Seed	Straw
T_1 - RDF (No fly ash)	0.054	0.06	0.19	0.06	0.09	0.05
T_2 - 20 t ha^{-1} Fly ash + RDF	0.072	0.10	0.29	0.10	0.14	0.08
T_3 - 40 t ha^{-1} Fly ash + RDF	0.076	0.10	0.31	0.22	0.14	0.12
T_4 - 60 t ha^{-1} Fly ash + RDF	0.096	0.19	0.34	0.28	0.15	0.15
T_5 - 80 t ha^{-1} Fly ash + RDF	0.071	0.08	0.28	0.09	0.13	0.06
T_6 - 100 t ha^{-1} Fly ash + RDF	0.054	0.07	0.22	0.07	0.10	0.05
T_7 - 120 t ha^{-1} Fly ash + RDF	0.062	0.09	0.25	0.10	0.11	0.07
SE (m) $\pm$	0.013	0.029	0.03	0.022	0.011	0.015
CD at 5%	-	0.081	0.09	0.063	0.031	0.042

Table 5. Effect of fly ash on oil content (%) in seeds of sunflower

Treatments	Oil content (%)
T_1 - RDF (No fly ash)	30.35
T_2 - 20 t ha^{-1} Fly ash + RDF	33.48
T_3 - 40 t ha^{-1} Fly ash + RDF	35.18
T_4 - 60 t ha^{-1} Fly ash + RDF	36.00
T_5 - 80 t ha^{-1} Fly ash + RDF	34.00
T_6 - 100 t ha^{-1} Fly ash + RDF	34.78
T_7 - 120 t ha^{-1} Fly ash + RDF	28.90
SE (m) $\pm$	0.93
CD at 5%	2.62

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e-planet **14** (2): 33-40 (December 2016)

Bioaccumulation of neodymium oxide (REE) and its effects on the growth and physiological changes of wheat and rice seedlings: A hydroponics study under plant growth chamber

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Date of receipt: 12. 09. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

A study of neodymium oxide (Rare earth element) on the seed germination and the growth of wheat and rice seedlings was carried out in Hoagland half strength solution containing 0.5, 2.0, 5.0, 10.0, 25.0 mg l^{-1} neodymium oxide (Nd_2O_3). A significant increase in germination and growth was observed with increased concentration of the aforesaid rare earth element(REE). Accumulation of neodymium (Nd) in shoot and roots parts of the plant were analyzed using ICP-OES. The bioaccumulation of Nd in the seedlings was positively correlated with the concentrations of metals in the culture medium and was higher in roots as compared to shoots. The absorbed water and imbibition process of the rice and wheat seeds increased during the soaking stage with Nd_2O_3, the plasma membrane permeability of the seeds increased, O_2 and H_2O were easier to get into the cell and the respiratory rate was enhanced. The role of REEs in promoting germination and growth can act as stimulating agents. Further research is suggested to study the effects of REEs on yields of agricultural crops.

Key words: Hydroponics study, neodymium oxide, rare earth elements, phytoremediation

INTRODUCTION

Increased urbanization and industrialization lead to develop new emerging technologies, especially within the consumer electronics, clean energy and military sectors. Since these sectors continue to rise so does the industry's demand for the constituent rare earth elements(REEs). The influx of rare earth elements (REEs) to the environment happen in many ways. One of the main features of REE is that the requirement does not grow uniformly for individual REEs, but rather depends on growth in the markets for derivative products of the individual REEs. As a result, our study only focused on members and parent oxides of these ten elements. In 1927, Neodymium compounds were first commercially marketed as glass dyes, and then remain as a popular additive in glasses. Due to the Nd^{3+} ion the colour of neodymium compounds is reddish-purple but it changes with the type of lighting, due to the interaction of the sharp light absorption bands of

neodymium with ambient light enriched with the sharp visible emission bands of mercury, trivalent europium or terbium. Neodymium is also used with different other substrate crystals, such as yttrium aluminum garnet in the Nd:YAG laser. Another significant use of Neodymium is as a constituent in the alloys used to make high-strength neodymium magnets, powerful permanent magnets which are widely used in commercial loudspeakers, microphones, in-ear headphones, and computer hard disks, where low magnet mass (or volume) or strong magnetic fields are indispensable. Huge neodymium magnets are used in high-power-versus-weight electric motors such as hybrid cars and generators such as aircraft and wind turbine electric generators.

Disposal of these wastes are much more hazardous than many other municipal wastes because electronic gadgets contain thousands of components made of deadly chemicals, heavy metals and Rare earth elements (REEs) that risk to human health and the environment. Since these sectors continue to raise so does the industry's demand. One of the key applications of REEs is in agriculture all over the world. Millions of tons of fertilizers that contain REEs are used worldwide for improving agricultural yield (Bremmer, 1994). The use of about 3.400 tons REEs over many hectares of cultivated lands in 2001 was reported in China (Source: China Rare Earth Information Center, Baotou, Inner Mongolia, China) though 50–100 million tons of REEs oxides entered the agricultural systems in 2001 according to Liang et al. 2005. Optimistic effects on crop production following treatments with REEs are largely reported in various literatures (Hu et al. 2004) and so many physiological responses have also been reported in diverse plant species (Fashui et al., 2000; Hu et al., 2002). Thus, The aim of this work was to study the effects of treatment with Nd_2O_3 on seed germination, seedling growth, protein content and antioxidant enzyme of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) in order to clarify the potential benefits or damages of Neodymium oxide (REEs) to plants.

MATERIALS AND METHODS

Nutrient solution preparation

For growth experiments Hoagland solution was used, it comprises of different macronutrients and micronutrients. Macronutrients comprises of 1M KH_2PO_4, 1M KNO_3, 1M Ca $(NO_3)_2$, 1M $MgSO_4$, 0.5% $FeCl_3$. Micronutrients comprises of 0.286% H_3BO_3, 0.022% $MnCl_2.4H_2O$, 0.002% $H_2MoO_4.H_2O$. The solution was adjusted to pH 6.5 with dilute HCl or NaOH and renewed every other day.

Preparation of stock solutions of neodymium oxide

Stock solution of five different concentrations of Neodymium oxide was used as treatment in this study were designated such as T_0 as control, T_1 as 0.5 mg l^{-1}, T_2 as 2.0 mg l^{-1}, T_3 as 5.0 mg l^{-1}, T_4 as 10.0 mg l^{-1} and T_5 as 25.0 mg l^{-1}. Neodymium and nutrient solution concentrations and pH values were measured and adjusted every two days as required. Nutrient solutions were stored at 24°C/ 18°C (day/ night) and relative humidity 60%/ 75% (day/ night).

Hydroponic-experimental design

Seeds of rice (*Oryza sativa* L., var. satyabhama) and wheat (*Triticum aestivum* L.) were collected from ICAR- National Rice Research Institute, Cuttack, Odisha. The seeds were surface sterilized with 0.5% (w/v) mercuric chloride solution for 5 min. The seeds were thoroughly washed under running tap water and rinsed several times with distilled water. The absorbent cotton was put on sterilized petriplates as bed along with filter paper to allow germination of seeds (10 seeds/ plate). Different concentrations of neodymium oxide viz. 0.5, 2.0, 5.0, 10.0, 25.0 mg l^{-1} were used along with control for germination. All experiments were carried out in triplicates in the growth chamber under controlled environment. After 24-48 hrs, germinated seeds were transferred to small containers containing Hoagland solution (half strength) and neodymium oxide of different concentrations for further study. Plants were grown under controlled environmental conditions with the help of plant growth chamber (Table 1 and Table 2).

Table 1. Physical conditions maintained in plant growth chamber during wheat (*Triticum aestivum* L.) growth under hydroponics

Day Time	Temperature	Light	Light
		Level (incandescent: Fluorescent)	Intensity (μE m^{-2} s^{-1})
9.00 am	18^0C	1:0	82
9.15 am	18^0C	1:2	291
10.00 am	18^0C	0:4	636
1.00 pm	23^0C	0:6	901
4.00 pm	22^0C	0:6	901
6.00 pm	20^0C	0:4	636
6.15 pm	20^0C	1:1	133
6.30 pm	20^0C	0:0	0

*Humidity at day time was 53% and at night it was 67%.
*Airflow from bottom to top (vertical current) was 14 meters per minute

Table 2. Physical conditions maintained in plant growth chamber during rice (*Oryza sativa* L.) growth under hydroponics

Day Time	Temperature	Light	Light
		Level (incandescent: Fluorescent)	Intensity (μE m^{-2} s^{-1})
9.00 am	23^0C	1:0	76
9.15 am	24^0C	1:2	280
10.00 am	32^0C	0:4	625
1.00 pm	34^0C	0:6	898
4.00 pm	32^0C	0:6	898
6.00 pm	34^0C	0:4	625
6.15 pm	34^0C	1:1	130
6.30 pm	28^0C	0:0	0

*Humidity at day time was 65% and at night it was 78%.
*Airflow from bottom to top (vertical current) was 14 meters per minute.

Growth and bio-chemical parameters

Plant growth was evaluated on the basis of root and shoot growth of the selected seedlings. Plant samples were harvested and washed with distilled water, and then cut into shoots and roots, and lengths were measured. Plant samples were dried at 85°C in an oven, the dry weights of roots and shoots were scored after 7, 15 and 21 d. Photosynthetic pigment of leaf content was measured following the method of Arnon (1949).

Neodymium(REE) analysis of plant tissue

Two plant samples were separated into root and shoot followed by rinsed with tap water and then Milli-Q water, dried at 105°C for 24 hours. The dried tissues were ground to powder. In aquaregia (HNO_3/ HCl, 1:3), the plant samples were

digested, and the concentration of Neodymium was determined using the Perkin Elmer Optima 2100 DV, ICP-OES (Inductively coupled plasma-Optical emission spectroscopy) after the adjustment of required dilution factor.

All the reagents and reference standards were of analytical grade from Himedia. Suprapure nitric acids and hydrochloric acid (Himedia) were used for sample digestion and preparation of standards.

RESULTS AND DISCUSSION

Seed germination

Different concentrations of neodymium had significant effect on seed germination. Similar observation was also observed by Aquino et.al in *Triticum durum* (Aquino et al., 2009). Wheat seeds showed best germination with 99% in 0.5mg l^{-1} neodymium oxide concentration right after 48 hrs of germination (Fig. 1).

Growth parameters

Effects of different concentrations of Nd_2O_3 on growth parameters were reported in Fig. 2-4. Stimulating effects of Nd on growth of both the plants was observed in T_3 as compared to other treatments and control one. With increase in concentrations (10 and 25 mg l^{-1} Nd_2O_3) there was a gradual decrease in growth of plants. It was consistent with the results of Diatloff et al. (1999) According to Chaturvedi et al., (2014) a positive effect of REEs was observed on shoot development of spinach and *Z. mays*. Zhang and Zhang also observed significant increase in growth of corn seedlings, thus improving yield. Neodymium oxide at all concentrations significantly reduced the dry weight of shoots and roots, except for treatments with 0.5, 2.0 and 5.0 mg/ l in the experiment (Fig. 1-2). The effects of Nd on plant growth may result from bioaccumulation, as they may change some metabolic functions of the plants. Hu et al. (2002) reported that La^{3+} and Ce^{3+} inhibit root elongation and reduce dry weight of roots and shoots in *Triticum aestivum* L. seedlings. Inhibitions of root elongation in barley (Van Stevenick et al., 1976) have been reported.

Effect of neodymium oxide on chlorophyll pigment content

Effect of Nd on photosynthetic pigments on rice and wheat was shown in Fig. 5 and 6. A significant positive effect on photosynthetic pigments was observed T_3 as compared to other treatments. Similar increase the chlorophyll content of corn was seen with concentrations up to 20mg l^{-1}, whereas decrease occurred with concentrations exceeding 50 mg l^{-1}. Furthermore, accelerated photosynthetic light reaction as well as growth promotion was also observed in tobacco (*Nicotiana tobacum*) seedlings after the treatment with 5-20 mg l^{-1} of lanthanum chloride (Chen et al., 2001). At concentrations of more than 15 mg kg^{-1} lanthanum, a decrease in chlorophyll contents as well as in chlorophyll a and b was observed in rape by Zeng et al., 2001. Hence lanthanum could also raise the Mg^{2+}-ATPase and so accelerate the Hill reaction in wheat (Sheng and Dai, 1994). Improved actions of both Mg^{2+}-ATPase and Ca^{2+}-ATPase on the chloroplast membrane were observed by Pan et al. (1998) after chloroplasts were treated with lanthanum chloride.

Physiologists have indicated that REEs can increase the activities of photosystem-II in plant and bind to its chlorophyll. It has also been reported that La accelerated the photosynthetic reactions at suitable concentration *in vivo* (Hong et al., 2001). Spraying rare earth on pepper foliars improved the total chlorophyll content of chlorophyll a and chlorophyll b (He et al., 1998). Therefore it may be concluded that the REEs have an intense effect on growth and pigment production in plants.

Effect of neodymium oxide on protein content

Effect of neodymium oxide on the activities of protein content of two plants was significantly increased (Fig. 7 and 8) in the T_3 than control but from T_4 it is decreasing which may be due the adverse effect of Nd on plants. The result indicated that T_0 has 60.9 mg g^{-1}, T_1 has 62.5 mg g^{-1}, T_2 has 63.5 mg g^{-1}, T_3 has 65.7 mg g^{-1}, T_4 has 55.7 mg g^{-1}, T_5 has 54.7 mg g^{-1} of protein content in

wheat plants and in case of rice plant T_0 has 60.5 mg g^{-1}, T_1 has 61.3 mg g^{-1}, T_2 has 64.0 mg g^{-1}, T_3 has 64.5 mg g^{-1}, T_4 has 57.1 mg g^{-1}, T_5 has 53.0 mg g^{-1} of protein content.

Effect of neodymium oxide on antioxidant enzyme content

Enzymes play a vital role in lowering the ROS levels and helping avoid oxidative stress. Effect of Neodymium on the activities of various antioxidant enzymes such as SOD, CAT, and POD was presented in Fig 7 and 8. Significant increase in the activities of enzyme content was observed in the treatments containing 5 mg l^{-1} (T_3). Role of lanthanides in the regulation of antioxidant enzymes has already been reported (Zhang et al. 2003; Jia et al. 2005; Ippolito et al. 2007) and the increase in the activity has also been proposed as an explanation for beneficial effects induced by lanthanides on aged seed germination (Fashui et al. 2000; Fashui, 2002).

Antioxidant enzymes are potential scavengers of H_2O_2 which minimize the oxidative and other stress in plant (Pandey et al. 2005).

Bioaccumulation of neodymium in the seedlings

Accumulation of Nd in shoots and roots was actually related to the concentrations of them in the treatments. With increase in concentrations of Nd from 0.5 to 25 mg l^{-1}, there was gradual increase in accumulation in shoots and in roots. The total amount of Neodymium accumulated in root is higher in compare to shoot after 30 days by ICP-OES analysis. It might be due to the fact that the transport from roots to shoots was much slower than their uptake. This was consistent with other results that considered roots as a barrier to the translocation of heavy metals to shoots (Zhang et al., 1999).

CONCLUSION

In conclusion the present findings affirm that the applications of Neodymium oxide in moderate doses of up to 5.0 mg l^{-1} can stimulate growth in all selected crops. Furthermore, wheat showed better tolerance towards Nd treatments than in rice. These results coincide well with reports of successful use of rare earths as fertilizers or plant growth stimulators in China and some European countries as well. Along with performance enhancing effects, rare earths also present low toxicity, even in long-term feeding trials. Although there is no confirmation to prove that REEs are necessary for plants to grow, many studies have suggested that REEs can stimulate plants to absorb, transfer and assimilate nutrients.

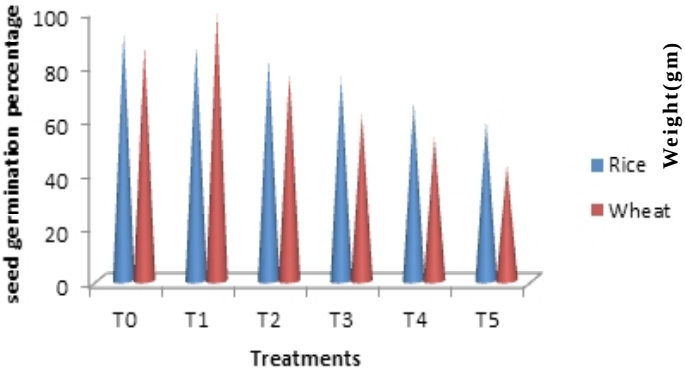

Fig. 1. Germination of both the seeds in percentage

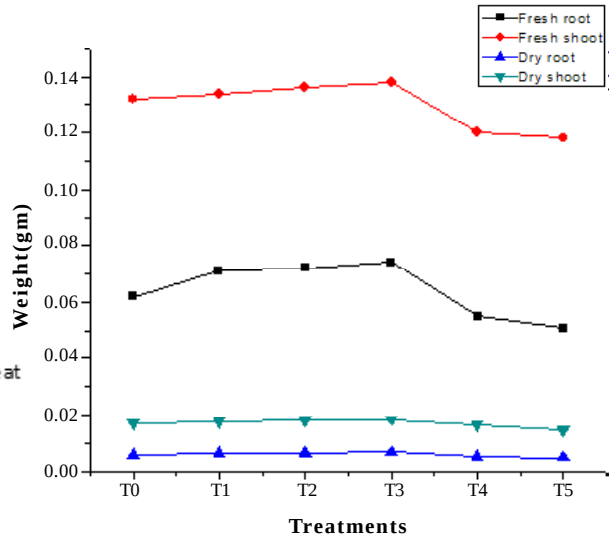

Fig. 2. Effect of Nd on growth of *Triticum aestivum*

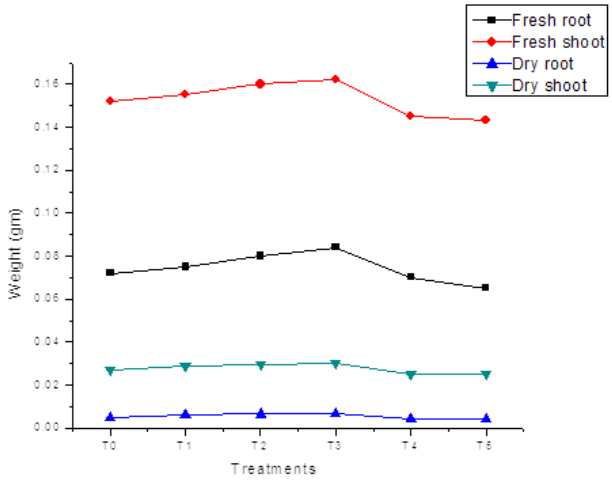

Fig. 3. Effect of Nd on growth of *Oryaza sativa*

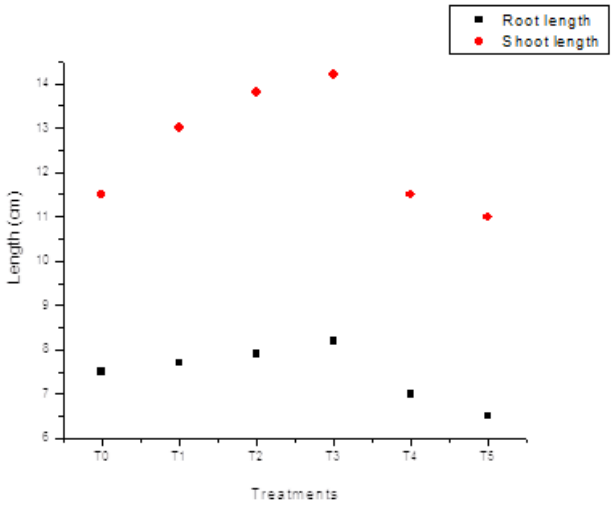

Fig. 4. Effect of Nd on root and shoot length of *Triticum aestivum*

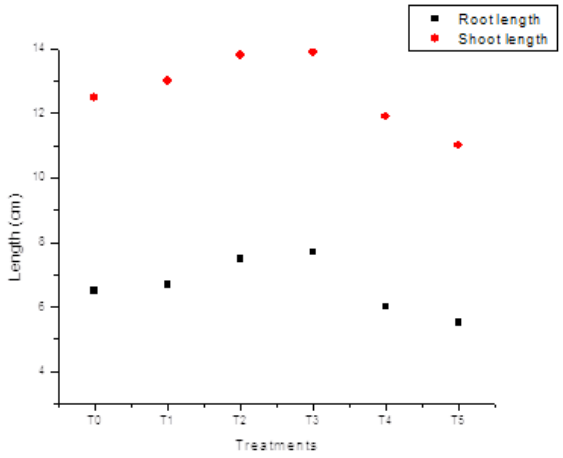

Fig. 5. Effect of Nd on root and shoot length of *Oryza sativa*

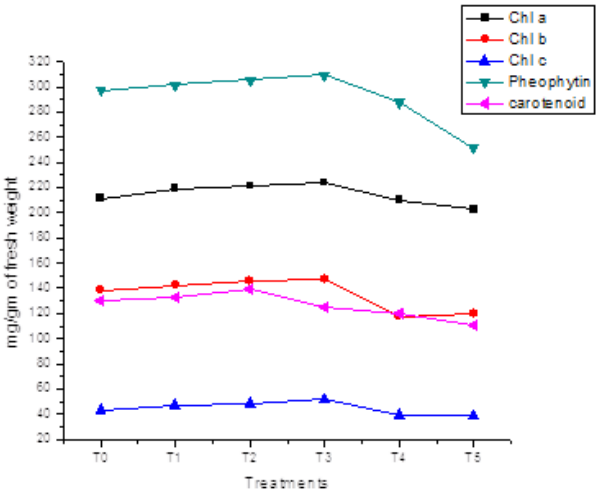

Fig. 6. Effect of Nd on photosynthetic pigment of *Triticum aestivum*

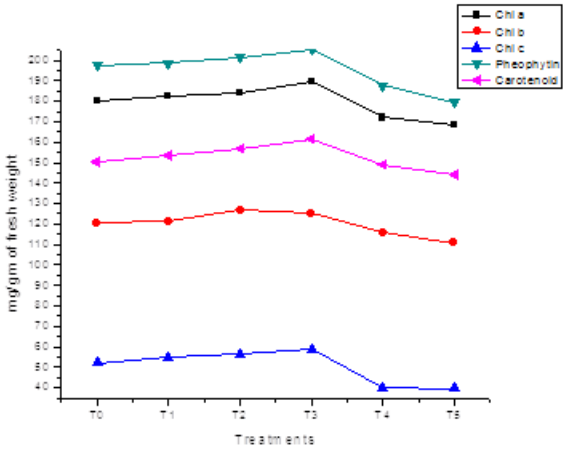

Fig. 7. Effect of Nd on photosynthetic pigment of *Oryza sativa*

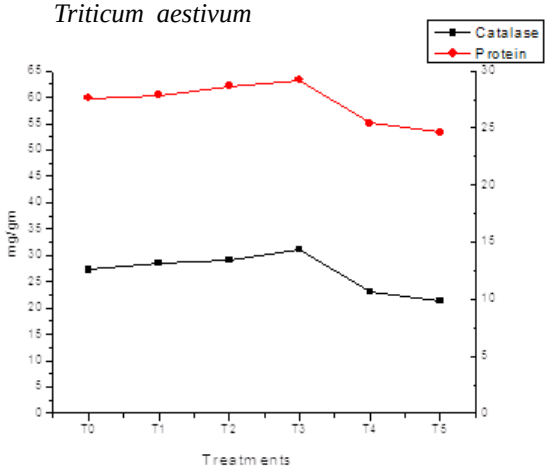

Fig. 8. Effect of Nd on protein and enzyme content of *Triticum aestivum*

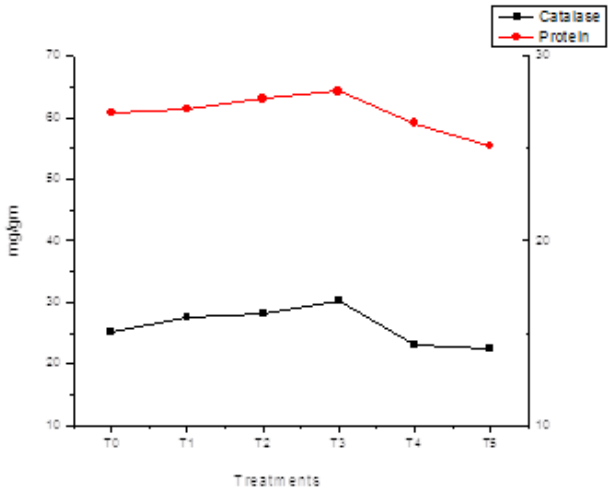

Fig. 9. Effect of Nd on protein and enzyme content of *Oryza sativa*

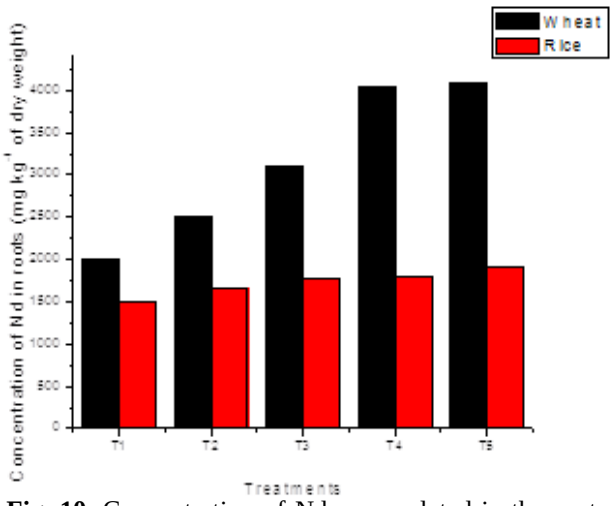

Fig. 10. Concentration of Nd accumulated in the roots of *Triticum aestivum* and *Oryza sativa*

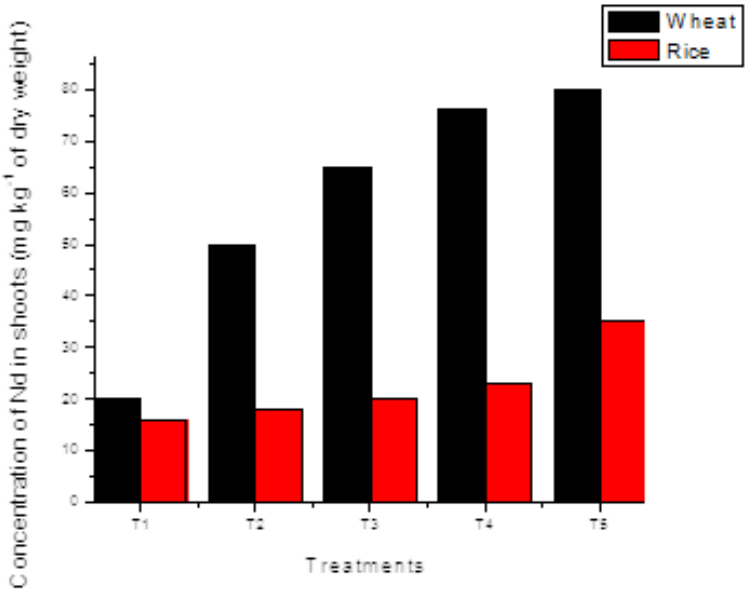

Fig. 11. Concentration of Nd accumulated in the shoots of *Triticum aestivum* and *Oryza sativa*

In addition, the worldwide use of Nd and other REEs as fertilizers in addition to feed additive supports the proposal of using plants to extract and remediate REE. As a result remediation and extraction of rare earths using suitable plants might be of interest as effective, safe and inexpensive alternative especially in developing countries.

ACKNOWLEDGEMENT

The authors are thankful to Prof. B.K. Mishra, Director, Institute of Minerals and Materials Technology, Bhubaneswar for providing necessary facilities to carry out the work and Council of Scientific and Industrial Research, (CSIR) New Delhi for financial support.

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e-planet **14** (2): 41-44 (December 2016)

Residual effect of S, Zn, B and FYM on yield and economics of summer rice (*Oryza sativa*)

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Date of receipt: 05. 08. 2016 Date of acceptance: 25. 11. 2016

ABSTRACT

A field experiment was conducted in lateritic soil of Instructional Farm, College of Agriculture, Bhubaneswar, Orissa University of Agriculture and Technology (OUAT) during summer season of 2010.-11 and 2011-12 to find out the residual effect of nutrient management practices on rice cv. '*Lalat*'. The soil was sandy loam in texture with acidic pH (5.8), medium in available N, P and K content and deficient in available S, Zn and B. The experiment was laid out in randomized block design with three replications and eleven treatments. During summer season, recommended fertilizer dose (RFD) @ 80-40-40 kg N-P_2O_5-K_2O ha^{-1} was given uniformly to all treatments where as S, Zn, B and farm yard manure (FYM) were applied with RFD in *wet* season. Their residual effect on yield and economics was studied in rice during summer season. Residual effect of $ZnSO_4$ @ 25 kg ha^{-1} + B @ 1 kg ha^{-1} produced the longest (28.1 cm) panicle, maximum number of panicles m^{-2} (331), more fertile grains $panicle^{-1}$ (166) and the highest test weight (24.58g). The same treatment recorded maximum grain yield (6.21 t ha^{-1}) and straw yield (7.45 t ha^{-1}) followed by residual effect of Zn-EDTA + S + B giving the second highest values of yield attributes and yield. Further, residual effect of $ZnSO_4$ + B recorded the highest gross return (Rs 74231 ha^{-1}), net return (Rs 47,231 ha^{-1}) and B-C ratio (1.75) followed by residual effect of Zn-EDTA + S+B. Residual effect of $ZnSO_4$ and FYM remained *at par* in terms of yield, gross return, net return and B-C ratio, indicating application of $ZnSO_4$ @ 25 kg ha^{-1} could be preferred over application of FYM @ 5 t ha^{-1}.

Key words: Sulphur, zinc, boron, farm yard manure, economics, rice

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal of Odisha. The state productivity (1815 kg ha^{-1}) is 34% less than national productivity of 2424 kg ha^{-1}(Anonymous, 2014). Growing of high yielding hybrid varieties and rise in cropping intensity with use of high input of N,P and K fertilizers with least or no organic manure has lead to rapid depletion of secondary and micro-nutrients in the soil. This has resulted in deficiency of S (28%), Zn (19%) and B (44%) in the soils of Odisha (Jena et al., 2008). The decline of rice productivity of the state may be attributed to these secondary and micronutrient deficiency in rice.

The cost of inputs, mainly fertilizers is increasing day by day. Therefore, emphasis is needed to maximize nutrient use efficiency and grain yield along with minimum cost of production (Pandey et al., 2007).

The efficiency of nutrients is raised by combined application of organic sources, fertilizer and slow release of micronutrients through organic ligands. Hence, a proper balance of organic sources of plant nutrients along with fertilizer and micronutrient was tried in rainy season to find out the residual effect of nutrient management practice in summer rice.

MATERIALS AND METHODS

Field experiments were conducted in lateritic sandy loam soil with acidic pH (5.8) at Instructional Farm, OUAT, Bhubaneswar during dry seasons of 2010-11 and 2011-12. The status of available N (295 kg ha^{-1}), P (15 kg ha^{-1}) and K (162 kg ha^{-1}) were medium, however, S (8 ppm), Zn (0.33 ppm) and B (0.43 ppm) content were deficient. The design of the experiment was randomized block design with eleven treatments replicated thrice with a plot size of 5m x 4m. The sources of different nutrients were N (urea), P (di-ammonium phosphate), K (Muriate of potash), S from Fertisulph-G (90% elemental S) and Zn from $ZnSO_4$ (23% Zn) and Zn ethylene di-amine tetra acetic acid (Zn- EDTA containing 12% Zn) along with B from borax (10.5% B). The N, P_2O_5 and K_2O content of FYM were 0.61%, 0.31% and 0.53%, respectively.

The treatments comprised of T_1- Recommended Fertilizer Dose (RFD) @ 80-40-40 kg N-P_2O_5-K_2O ha^{-1} T_2- RFD + farm yard manure (FYM) @ 5 t ha^{-1}, T_3- RFD + $ZnSO_4$ @ 25 kg ha^{-1}, T_4-RFD + Zn-EDTA @ 1 kg ha^{-1} , T_5-RFD + S @ 30 kg ha^{-1}, T_6-RFD + B @ 1 kg ha^{-1}, T_7-RFD + Zn-EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1}, T_8-RFD + Zn-EDTA @1 kg ha^{-1}+B @1 kg ha-^{1}, T_9- RFD + S @ 30 kg ha^{-1} + B @ 1 kg/ha^{-1} ,T_{10}-RFD + $ZnSO_4$ @ 25 kg ha^{-1} + B @ 1 kg ha^{-1} and T_{11}-RFD + Zn-EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1}. Only S, Zn, B and (FYM) were applied to designated treatments in wet season and its residual effect was studied in dry season on yield and economics of summer rice cv *'Lalat'* fertilized with uniform dose of RFD(80-40-40 kg N-P_2O_5-K_2O ha^{-1}) only. The duration of the test variety was 130 days and it was planted at row spacing of 15 cm and plant to plant spacing of 10 cm with two seedlings $hill^{-1}$. The benefit: cost ratio was computed by dividing the net return with cost of cultivation (Sing et al.,2015).

RESULTS AND DISCUSSION

Yield and yield attributes

The yield and yield attributes of summer rice were significantly influenced by residual effect of nutrient management treatments being applied in wet season (Table 1 and 2). The residual effect of $ZnSO_4$ @ 25 kg ha^{-1} + B @ 1 kg ha^{-1} produced longest panicle (28.1 cm), maximum panicles m^{-2} (331), more fertile grains $panicle^{-1}$(166) and the highest test weight (24.58 g). The same treatment in residual effect recorded maximum pooled grain (6.21 t ha^{-1}) and straw (7.45 t ha^{-1}) yield. This was closely followed by the residual effect of Zn-EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1} giving the next highest values of yield attribute and yield. Considering the residual effect of two sources of Zn with RFD, $ZnSO_4$ @ 25 kg ha^{-1} applied to *Kharif* rice recorded higher grain yield (5.48 t ha^{-1}) than Zn-EDTA @ 1 kg ha^{-1} (4.59 t ha^{-1}). Residual effect of $ZnSO_4$ also recorded more grain yield than residual effect of FYM (5.38 t ha^{-1}) which remained at par .

The productive panicles m^{-2} and panicle length were at par with the residual effect of $ZnSO_4$+B and Zn-EDTA+S+B. But the residual effect of $ZnSO_4$ and B recorded the highest number of fertile grains $panicle^{-1}$ and test weight being significantly superior over residual effect of Zn-EDTA+S+B. Residual effect of $ZnSO_4$+B exhibited favorable effect on yield attributes that might be due to better absorption/ availability of Zn, S and B in adequate amount throughout the growth period and their synergistic effect in improving yield components.

Pooled data (Table-2) revealed that the productivity of rice was significantly the highest in residual effect of $ZnSO_4$ + B (6.21 t ha^{-1}) over residual effect of Zn-EDTA + S + B (5.66 t ha^{-1}) and $ZnSO_4$ (5.48 t ha^{-1}). Residual effect of $ZnSO_4$ + B recorded 13 and 38 per cent increase in yield over residual effect of $ZnSO_4$ and B. Residual effect of FYM recorded an increase of 26, 17,14 and 20 per cent in grain yield over residual effect of RFD, Zn-EDTA, S and B, respectively. Organic sources of plant nutrients (FYM) applied in wet season benefitted the succeeding summer rice due to its slow release of nutrients for a longer period resulting in higher grain yield as compared to sole application of S/B/Zn-EDTA and their combinations. Residual effect of $ZnSO_4$ + B recorded 45 per cent increase

in grain yield over RFD. The straw yield (Table - 2) recorded due to imposition of residual effect of various treatments in summer season followed the similar trend as that of grain yield. It was observed that residual effect of $ZnSO_4$+ B enhanced the yield remarkably as the inherent Zn status of the soil (0.33 ppm) was very low and below optimum level (0.6 ppm). Sujathamma et al.(2013) have reported higher yield due to residual effect of $ZnSO_4$ in rice-rice cropping system.

Increase in grain and straw yield due to residual effect of $ZnSO_4$ + B compared to no Zn/ B application might be due to better utilization of N and P resulting in increased metabolic activities. Further, it contained higher amount of zinc with more solubility thus, improved fertilizer use efficiency in residual condition compared with sole application of $ZnSO_4$/ S/ B and Zn-EDTA and their combinations. Higher yield due to residual effect of $ZnSO_4$ + B might be attributed to various enzymatic reactions and catalytic effect on growth process and hormone production during protein synthesis (Channabasavanna et al., 2001).

Moreover, residual effect $ZnSO_4$ + B favored root growth, with mobilization of plant nutrients at optimum levels and there by increased grain and straw yield of rice (Das and Singh, 1982). It might be due to effect of residual Zn on uptake of extra plant nutrients i.e., (N, P, K, Zn, S and B) though enzymatic action in metabolic process which resulted in higher grain yield. Boron and zinc are known to influence translocation of metabolites and improving source and sink strength in plants (Mauriya et al., 2013). This might caused higher yield attributes and yield in *rabi* rice. Further, residual effect of B due to imposition of $ZnSO_4$ + B during *wet* season increased fertilization of flowers which had a favorable effect on grain yield.

ECONOMICS

The residual effect of $ZnSO_4$ + B registered the maximum gross return (Rs.74,231 ha^{-1}), net return(Rs 47231 ha^{-1}) and B:C ratio(1.75) followed by residual effect of Zn-EDTA + S + B (Table 2). It was possible due to greater yield advantage and better fertilizer use efficiency by residual effect of $ZnSO_4$+ B over all other treatments. While comparing cost effectiveness between application

Table 1. Residual effect of FYM, S, Zn and B on yield attributes of summer rice (pooled of two years).

Treatments		Number of panicles m^{-2}	Panicle length (cm)	Fertile grains $panicle^{-1}$	1000-grain weight (g)
T_1	RFD (80-40-40kg N-P_2O_5-K_2O ha^{-1})	258	21.8	107	21.75
T_2	RFD + FYM @ 5 t ha^{-1}	308	25.1	141	24.05
T_3	RFD + Zn SO_4 @ 25 kg ha^{-1}	319	26.5	152	24.25
T_4	RFD + Zn EDTA @ 1 kg ha^{-1}	278	22.4	117	22.88
T_5	RFD + S @ 30 kg ha^{-1}	283	22.5	122	23.20
T_6	RFD + B @ 1 kg ha^{-1}	272	22.3	114	22.65
T_7	RFD + Zn EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1}	301	23.7	137	24.00
T_8	RFD + Zn-EDTA @ 1 kg ha^{-1} + B @ 1kg ha^{-1}	290	22.7	126	23.72
T_9	RFD + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1}	293	23.1	132	23.90
T_{10}	RFD + $ZnSO_4$ @ 25 kg ha^{-1} + B @ 1 kg ha^{-1}	331	28.1	166	24.58
T_{11}	RFD + Zn-EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1}	325	28.0	158	24.38
SEm (±)		3.80	0.07	1.25	0.03
CD (P = 0.05)		11.40	0.21	4.16	0.09

of RFD+$ZnSO_4$+ B and RFD+Zn-EDTA+S+B, it was observed that the residual effect of former treatment is preferred due to the lower cost of $ZnSO_4$(Rs 45/kg) than Zn- EDTA (Rs 790/kg) .

It was observed that gross return, net return and B:C ratio recorded with residual effect of $ZnSO_4$ remained at par with residual effect of FYM. It may be perceived that due to low cost of commercial grade of $ZnSO_4$ and its better effectiveness in improving yield, application of $ZnSO_4$ maybe advocated for higher return instead of use of FYM which costs more. Sujathamma et al., 2013 have reported higher economic returns due to residual effect of $ZnSO_4$ in rice-rice cropping system.

CONCLUSION

The residual effect of $ZnSO_4$ @ 25 kg ha^{-1} + B @1 kg ha^{-1} in summer rice cv '*Lalat*' recorded maximum values of yield attributes and registered the highest grain yield, net return and B:C ratio over Zn-EDTA @1.0 kg ha^{-1}+ S @30 kg ha^{-1}+ B @1.0 kg ha^{-1}.

Table 2. Residual effect of FYM, S, Zn and B on yield and economics of summer rice (pooled of two years).

Treatments		Grain yield (t ha^{-1})	Straw Yield (t ha^{-1})	Gross return (Rs ha^{-1})	Net return (Rs ha^{-1})	B:C ratio
T_1	RFD (80-40-40kg N-P_2O_5-K_2O ha^{-1})	4.28	6.11	51920	24920	0.92
T_2	RFD + FYM @ 5 t ha^{-1}	5.39	6.72	64515	37515	1.39
T_3	RFD + Zn SO_4 @ 25 kg ha^{-1}	5.51	6.81	65728	38728	1.43
T_4	RFD + Zn EDTA @ 1 kg ha^{-1}	4.58	6.14	55346	28346	1.05
T_5	RFD + S @ 30 kg ha^{-1}	4.70	6.25	57055	30055	1.11
T_6	RFD + B @ 1 kg ha^{-1}	4.44	6.09	54126	27126	1.00
T_7	RFD + Zn EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1}	5.20	6.64	62619	35619	1.32
T_8	RFD + Zn-EDTA @ 1 kg ha^{-1} + B @ 1 kg ha^{-1}	4.94	6.41	59227	32227	1.19
T_9	RFD + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1}	5.09	6,50	61071	34071	1.26
T_{10}	RFD + $ZnSO_4$ @ 25 kg ha^{-1} + B @ 1 kg ha^{-1}	6.28	7.45	74231	47231	1.75
T_{11}	RFD + Zn-EDTA @ 1 kg ha^{-1} + S @ 30 kg ha^{-1} + B @ 1 kg ha^{-1}	5.63	6.95	67811	40811	1.51
	SEm (±)		0.06	579	843	0.02
	CD (P = 0.05)		0.16	1707	2529	0.06

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e-planet **14** (2): 45-56 (December 2016)

Wild edible fruits traditionally used by tribes of Similipal Biosphere Reserve, Odisha, India

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Date of receipt: 10. 09. 2016 Date of acceptance: 11. 11. 2016

ABSTRACT

The present study was carried out in Similipal Biosphere Reserve, Odisha to assess the diversity and consumption pattern of wild edible fruit plants sustained by local tribal inhabitants. The study was based on extensive botanical survey, interview with traditional knowledge holders and documented information on indigenous traditional knowledge of major tribes of selected 30 villages of Similipal Biosphere Reserve. Altogether species diversity of 92 wild edible fruit plants belonging to 41 families and 67 genera were documented along with their local name, mode of consumption and income generating species maintained by the local tribes.

Key words: Wild edible fruits, species diversity, consumption pattern, Similipal Biosphere Reserve

INTRODUCTION

From the time immemorial, wild edible plants of forested landscape have been playing a vital role by supplementing nutrition and diet to the tribal communities (Misra et al., 2013). Primitive man in search of food had consumed various plant parts such as roots, tubers, leaves, fruits and nuts collected from the wild, before he learnt to grow plants. Living in nature, man screened out plants those were edible and identified plants unsuitable for consumption. Among the forest food plants, wild fruits play a crucial role in supplementing the food requirements of tribal people in remote areas.

Besides the few cultivated fruit species, there are many others those growing wild in forest habitats of our country are neglected/ underexploited, and their economic potential is still unknown. These fruits are nutritionally rich and can supplement nutritional requirements especially micronutrients and vitamins to the human beings (Maundu et al., 1999). With the advent of modern agriculture in India, however, scant attention has been paid to exploit the genetic resources of these wild fruits which are particularly significant in the face of severe genetic erosion leading to extinction of valuable species on account of forest degradation, urbanization and intensive agriculture.

It has been estimated that there are more than 3000 edible fruits and nuts in the tropics of both the hemispheres (Mahapatra and Panda, 2009). The Indian subcontinent is bestowed with enormous diversity of wild fruits in its wide range of agro-ecological zones throughout the tropical, sub-tropical and temperate regions. There are about 647 species of which more than 100 species are more agreeable

types (Arora and Pandey, 1996). The wild fruits of tropical/ subtropical tracts are mainly from the members of families of Anacardiaceae, Annonaceae, Clusiaceae, Euphorbiaceae, Moraceae, Myrtaceae, Rutaceae, Tiliaceae etc. Although certain species occur predominantly in the tropics and sub-tropics, semi-arid and arid tracts, such as Deccan plateau, Eastern and Western Ghats, few species like *Aegle marmelos, Alangium salvifolium, Mangifera indica, Phyllanthus emblica, Limonia acidissima, Artocarpus lacucha, Syzygium cumini, Tamarindus indica, Ziziphus* spp., *Carissa* spp. are commonly found throughout the Indian subcontinent. Genetic diversity is very rich in the north-western Himalayas and to relatively low extent in the north-eastern region.

On account of varied vegetation types and rich flora, the Similipal Biosphere Reserve, the representing ecosystem in Eastern India, harbours rich diversity of wild fruit plants which serve as food supplements, nutritionally balanced diets and contribute to the economy of forest dwellers and rural inhabitants. Despite its rich diversity and economic potentiality, no attempt has been made to assess the diversity of plant species and consumption pattern of this group. Therefore, an inventory of such wild edible fruit plants was made by field survey and means of interviewing rural/ tribal communities of this region regarding the traditional use of such resources.

MATERIALS AND METHODS

Study site and aboriginal tribes

The study was conducted in Similipal Biosphere Reserve in northern Odisha, the representative ecosystem under Mahanadian biogeographic region of tropical eastern India and one of the biodiversity hot spot of India. The biosphere reserve has varied topography, rich biological biodiversity and habitat of aboriginal/ primitive types. The reserve spreads over an area of 5569 sq km and lies between 21^0 10' to 22^0 12' N latitude and 85^0 58' to 86^0 42' E longitude (Fig.1) ranging between 200m to1178 m above mean sea level. Its administrative forest divisions are Baripada, Karanjia and Raiangpur which are close to the borders of Jharkhand and West Bengal. The Similipal hills drain mostly eastwardly by a number of perennial streams which ultimately join with main river systems such as the Budhabalang, the Baitarani and the Subarnarekha those finally drain into the Bay of Bengal. Most of the streams pass through the undulating hills giving rise to a number of magnificent waterfalls such as Barheipani and Joronda and few small others. The Similipal prevails tropical monsoonal climate. Summer is tolerable as the temperature hardly goes above 40^0. The average annual rainfall of the Similipal Biosphere Reserve ranges from 1800 to 2900 mm precipitation in 135- 158 days annually (Misra, 2006). High relative humidity prevails throughout the year which goes up to 90 per cent during rainy season.

Similipal Biosphere Reserve harbours a very rich flora comprising 1254 species of vascular plants representing 46 per cent of the flora of Odisha and 7 per cent that of India including 94 species of orchids and 52 species of rare/ endangered plants (Misra et al., 2011; Misra, 1997; Mishra, 1989). It has a unique biodiversity possessing a number of endemic, threatened, medicinal and economic plants, 23 species of bryophytes and a centre of origin and diversification for a significant number of crop plants and their wild relatives (Paroda and Arora, 1987; Saxena and Brahmam, 1989; Misra, 1997; Dash et al., 2007).

Similipal forests can rightly be called as the “abode of aboriginal and primitive tribes”. It is a unique forest ecosystem that provides the local tribes with their essential needs of life. The scheduled tribes such as Kolha, Santhal, Bathudi, Bhumija, Gonda are dominant including Khadia and Mankirdia as primitive ones and the tribal population constitutes 74 per cent of total population of Similipal. Their main occupation is food gathering, hunting, collection of forest products and traditional farming. Though the bulk of their current diet is rice they mostly otain many food items from the forest as a substitute of regular/ part time diet, especially in times of food scarcity (Misra and Bal, 2007).

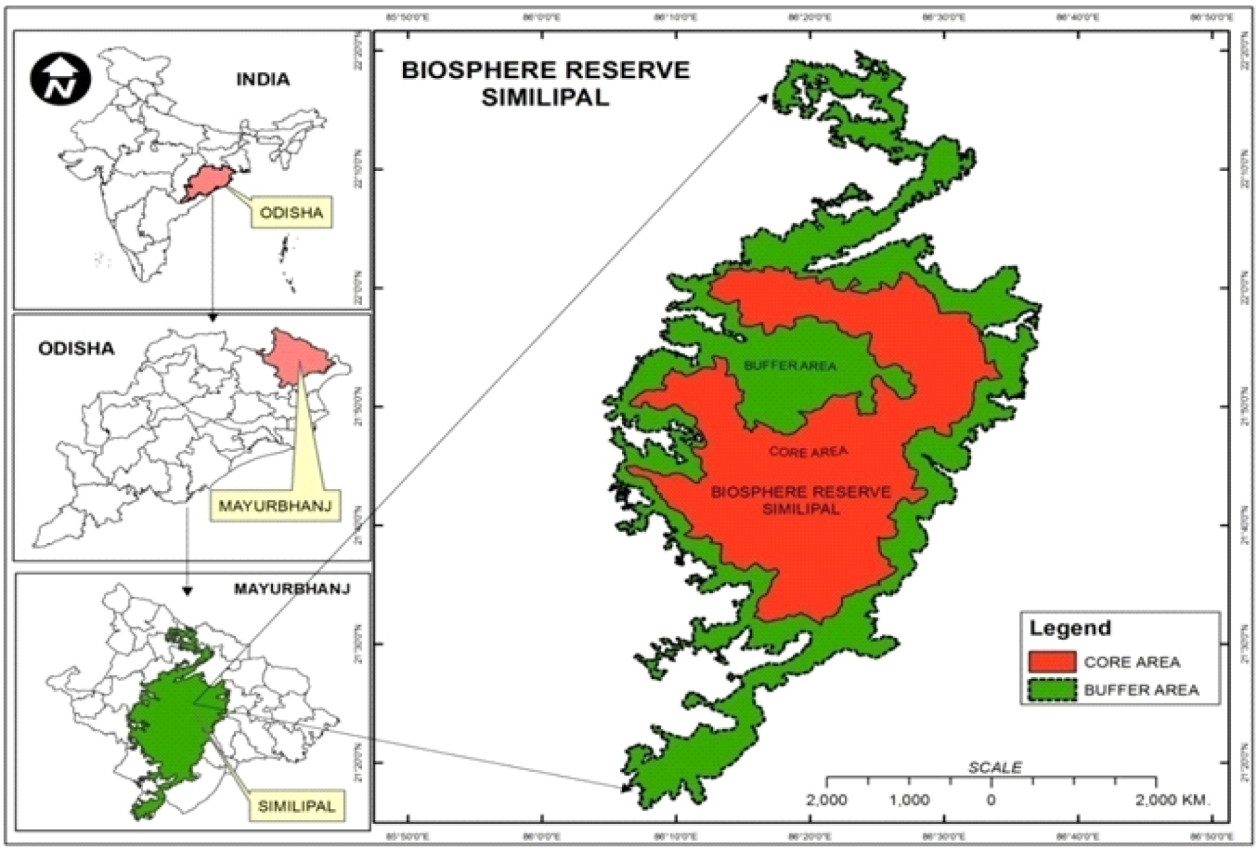

Fig. 1. Location map of Similipal Biosphere reserve

In this study, efforts have been made to document the diversity of species and mode of consumption of wild edible fruits of Similipal Biosphere Reserve. Survey, inventory, collection and assessment of wild fruits were conducted during 2008-2013. The standard participatory rural appraisal method (Cunningham, 2001; Gerique, 2006) was adopted for sampling and data collection to document the indigenous traditional knowledge on edible fruit plants. Primary data was collected through botanical survey, structured interview of households, group discussions and market survey. A total of 157 informants of different age groups (8 to 65 years) of four tribal communities from 30 villages were interacted using snow ball sampling technique on indigenous uses of edible fruit plants with the assistance of community knowledge holders and local forest officials. Botanical surveys were conducted in different landscapes of diverse forest types, homesteads and farm lands and the details of edible uses of fruit plants occurring in different habitats were recorded. Information on diversity of fruit species used as food along with their mode of consumption was gathered and botanical names were assigned immediately on the spot.

RESULTS AND DISCUSSION

An inventory of wild edible fruit plants was made by field survey and means of interviewing the tribal households of this region regarding the indigenous use of such resources. The tribal communities of Similipal have a long history of wild fruit gathering, many of them are eaten raw when ripe whereas few species are consumed during times of food scarcity. The unripe fruits of some species are also burnt or cooked into vegetables or pickled and kept for many days to eat with day

meals facilitating them instantly when they leave for full day hard work. Sometimes few species are also used as beverages/ cooling drinks. Many of these fruits are delicious, provide instant energy and meet food requirements to some extent.

Taxonomic diversity

The investigation revealed that a total of 92 wild edible fruit plants belonging to 40 families and 67 genera were found to be used as food by the inhabitants of Similipal Biosphere Reserve (Table 1). These species are distributed over 84 dicotyledons, 6 monocotyledons and 2 gymnosperms.

Among the documented wild fruit species, the most dominant families taken into account are Moraceae (7), Rutaceae (6), Annonaceae (5), Euphorbiaceae (5), Rhamnaceae (5), Rubiaceae (5). The other families show low representatives of fewer species (Fig. 2).

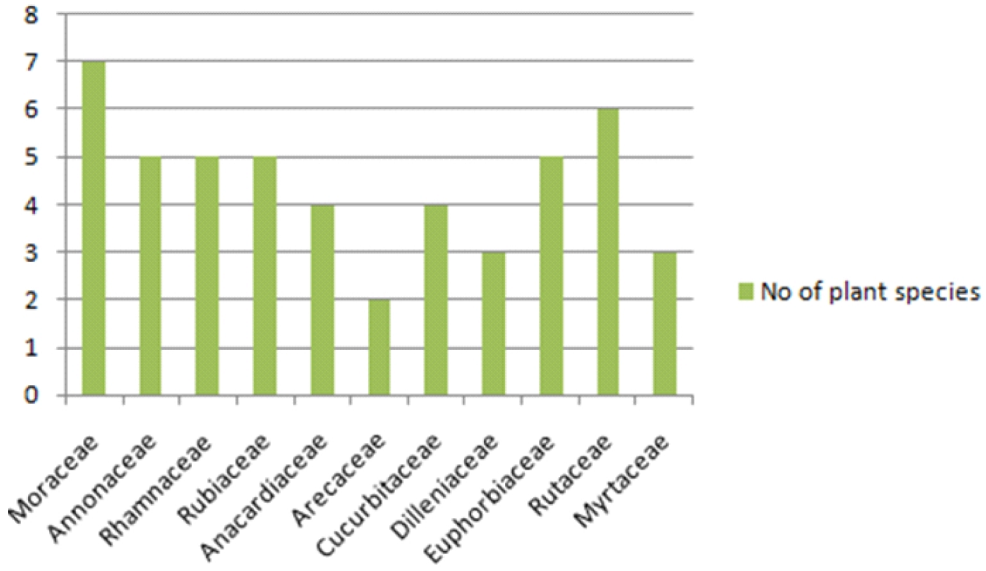

Fig. 2. Dominant families with number of plant species

Growth form

The growth form of wild edible fruit species comprises of trees, climbers/ lianas, shrubs and herbs. Out of 92 species of documented wild fruit plants, 57 species (62 per cent) are trees followed by 17 species of climbers/ lianas (19 percent), 15 species of shrubs (16 per cent) and 3 species (3 per cent) are herbs (Fig. 3).

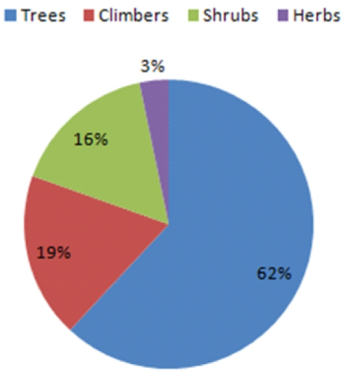

Fig. 3. Growth form of wild edible fruit species

Table 1. Species diversity, fruiting form and consumption pattern of wild edible fruit plants

Sl. No	Name of family	Name of species	Local name	Fruiting form	Mode of consumption	Food item (s)	Frequency of intake
1	Anacardiaceae	*Buchanania lanzan*	Char (Kl)	Ripe kernel	Raw/roasted	Snacks/relishes	Frequent
2		*Mangifera indica*	Am(Ba)	Ripe, unripe	Raw, cooked	Snacks, curry/pickled	Frequent
3		*Semecarpus anacardium*	Bhalia(Ba)	Ripe hypocarp, kernel	Raw/burnt	Snacks	Normal
4		*Spondias pinnata*	Ambda(San)	Unripe, ripe	Cooked, raw, pickled	Snacks, curry pickled	Normal
5	Annonaceae	*Alphonsea lutea*	Chaunri muthi (Kh),Kare(Kl)	Ripe	Raw	Snacks	Occasional
6		*Alphonsea ventricosa*	Nuha jhadi(Kh)	Ripe	Raw	Snacks	Occasional
7		*Polyalthia cerasoides*	Tapa (Kl)	Ripe	Raw	Snacks/relishes	Normal
8		*Miliusa tomentosa*	Dom sala(Kl)	Ripe pulp	Raw	Snacks/relishes	Occasional
9		*Uvaria hamiltonii*	Sati phal (Kh)	Ripe	Raw	Snacks	Normal
10	Arecaceae	*Phoenix acaulis*	Pala (Kh)	Ripe	Raw	Snacks	Frequent
11		*Phoenix sylvestris*	Marang pala(Kl)	Ripe	Raw	Snacks	Frequent
12	Araceae	*Scindapsus officinalis*	Daru Japa(Kl), Dare japa(San)	Matured	Cooked	Curry	Occasional
13	Burseraceae	*Protium serratum*	Rimbidi Kandheiar (Kl)	Ripe	Raw	Snacks, Chutney	Normal
14	Caesalpiniaceae	*Bauhinia vahlii*	Siali(Kh)	Matured seed	Burnt	Snacks/relishes	Frequent
15		*Cassia fistula*	Sunari(San)	Matured seed	burnt	Snacks	Occasional
16	Capparaceae	*Capparis brevispina*	Nepheda(San)	Ripe	Raw	Snacks/relishes	Occasional

17		*Capparis zeylanica*	Asadhua(Ba)	Matured	Cooked	Curry	Normal
18	Clusiaceae	*Garcinia cowa*	Chiunr(Ba)	Ripe	Raw, pickled	Snacks	Normal
19		*Garcinia xanthochymus*	Kiunr(Kl)	Ripe	Raw, cooked	Snacks, curry	Normal
20	Combretaceae	*Terminalia bellerica*	Beheda(Kh)	Seed	Burnt	Snacks	Occasional
21		*Terminalia chebula*	Harda(Kh)	Kernel	Burnt	Snacks	Occasional
22	Convolvulaceae	*Erycibe paniculata*	Kari(San,Kl),Dur koli(Kh)	Ripe	Raw	Snacks/relishes	Occasional
23	Cucurbitaceae	*Gymnopetalum cochinchinense*	Koubutka(San)	Unripe	Cooked	Curry, fry	Normal
24		*Momordica dioica*	Buru kankada(Kh)	Unripe	Fried, Curry	Curry	Frequent
25		*Solena amplexicaulis*	Bana Kunduri	Unripe	Raw/fried/ vegetable curry	Snacks, curry	Normal
26		*Thladiantha cordifolia*	Buru karda	Unripe	Cooked	Curry	Normal
27	Cycadaceae	*Cycas circinalis*	Biru(Kl)	Ripe	Processed and cooked	Cake	Occasional
28	Dilleniaceae	*Dillenia aurea*	Bada rai(Kh)	Unripe, ripe	Cooked	Curry	Normal
29		*Dillenia pentagyna*	Rai(Kh)	Flower, calyx, ripe	Raw /cooked	Fry, curry	Normal
30		*Dillenia indica*	Oau(San)	Ripe	Cooked, pickled	Sour curry pickled	Frequent
31	Dipterocarpaceae	*Shorea robusta*	Sarjam(Kl)	Matured seed	Boiled with Mahua flower	Snacks	Occasional
32	Ebenaceae	*Diospyros embryopteris*	Mankad kendu(Ba)	Ripe	Raw	Snacks/ dessert	Frequent
33		*Diospyros melanoxylon*	Kendu(Ba)	Ripe	Raw	Snacks/ dessert	Frequent
34	Euphorbiaceae	*Antidesma acidum*	Mata (Kl)	Ripe	Raw	Snacks/relishes	Normal

35		*Antidesma ghaesembilla*	Surei Matha sag(Kl)	Ripe	Raw, cooked	Snacks, curry	Normal
36		*Baccaurea ramiflora*	Rajnjana(kh) Rajhada(Kh)	Ripe	Raw, cooked	Snacks, curry	Frequent
37		*Bridelia retusa*	Kasiphal(Kh)	Ripe	Raw	Relishes	Occasional
38		*Phyllanthus emblica*	Amla(Kh)	Ripe/matured	Raw, dried	Snacks	Frequent
39	Fabaceae	*Atylosia scarabaeoides*	Buru kolthi(Kh)	Ripe	Raw	Snacks	Occasional
40		*Butea superba*	Marda(Ba)	Ripe	Cooked	Snacks	Occasional
41	Flacourtiaceae	*Flacourtia indica*	Sana baincha(San)	Ripe	Raw	Snacks	Occasional
42		*Flacourtia jangomas*	Merle(Kl)	Ripe	Raw	Snacks	Occasional
43	Gnetaceae	*Gnetum ula*	Mirig lendi(Kl)	Seed	Fried	Snacks	Occasional
44	Lauraceae	*Litsea glutinosa*	Jaysandha(Kh)	Ripe	Raw	Snacks	Occasional
45	Lecythidaceae	*Careya arborea*	Kumbh	Unripe	Cooked	Curry	Occasional
46	Leeaceae	*Leea macrophylla*	Hati Hat Kan(San,Kl)	Ripe	Raw	Snacks	Occasional
47	Mimosaceae	*Pithecellobium dulce*	Sima kayan(Kh)	Ripe fruit aril	Raw	Relishes	Frequent
48	Moraceae	*Artocarpus lacucha*	Daucha (Kh)	Ripe	Raw/ cooked	Snacks, pickled, sour curry	Frequent
49		*Ficus auriculata*	Guda khari(Kl)	Ripe	Raw	Ripe	Occasional
50		*Ficus heterophylla*	Butihasa(Kl)	Ripe	Raw	Snacks	Normal
51		*Ficus hispida*	Dumer (Kl)	Ripe	Raw and cooked	Snacks, curry	Occasional
52		*Ficus recemosa*	Marang dumer (Kl)	Ripe	Raw and cooked	Snacks, curry	Occasional
53		*Ficus virens*	Dumer (Kl)	Ripe	Raw	Snacks/ relishes	Occasional

54		*Ficus semicordata*	Padhel (Kh)	Ripe	Raw	Snacks	Normal
55	Musaceae	*Musa paradisiaca var. sapientum*	Buru kadali(Ba)	Unripe	Cooked	Curry	Occasional
56	Myrsinaceae	*Ardisia solanacea*	Tinkoli (Kl) Jungle Guleicha (Kh)	Ripe	Raw	Snacks	Normal
57	Myrtaceae	*Sygyzium cerasoides*	Poi jam (Kh)	Ripe	Raw	Snacks/ dessert	Occasional
58		*Sygygium cumini*	Jam (San)	Ripe	Raw	Dessert	Frequent
59		*Syzygium fruticosum*	Kude daru(Kl)	Ripe	Raw	Snacks	Frequent
60	Orchidaceae	*Dendobium moschatum*	Daru janapa (Kl)	Unripe	Cooked	Curry	Occasional
61		*Dendrobium formosum*	Daru janapa (Kl)	Unripe	Cooked	Curry	Occasional
62	Passifloraceae	*Passiflora foetida*	Balbalua(Kh)	Ripe, unripe	Raw, cooked	Snacks, curry	Normal
63	Rhamnaceae	*Ziziphus funiculosa*	Chunkoli (Kl)	Ripe	Raw	Snacks	Occasional
64		*Ziziphus mauritiana var. fruticosa*	Buro koli(Kh)	Ripe	Raw, pickled	Snacks, curry relishes	Frequent
65		*Ziziphus oenoplia*	Kanto Koli(Ba, Kh)	Ripe	Raw	Snacks/dessert	Frequent
66		*Ziziphus rugosa*	Sirka Koli (Kl.)	Ripe	Raw	Snacks	Occasional
67		*Ziziphus xylopyra*	Karkata(Kl)	Ripe	Raw	Snacks	Occasional
68	Rosaceae	*Rubus ellipticus*	Kandheikoli (Kh),Nai tunt koli(Kl)	Ripe	Raw	Snacks	Normal
69	Rubiaceae	*Canthium dicoccum*	Jur Daru(Kl) Chianr (kh)	Ripe	Raw	Snacks/ relishes	Occasional
70		*Catunaregam spinosa*	Sharli (Kl)	Ripe/ matured	Cooked	Curry	Normal
71		*Gardenia gummifera*	Ghurudu	Ripe	Raw	Snacks	Frequent
72		*Gardenia latifolia*	Papla(Kl)	Ripe	Raw	Snacks	Normal

73		*Tamilnadia uliginosa*	Sarli	Unripe	Cooked	Curry	Occasiona
74	Rutaceae	*Aegle marmelos*	Bela	Ripe	Raw, drink, pickled	Snacks, Juice, sherbet	Frequents
75		*Atalantia monophylla*	Narguni(San)	Matured	Pickled	Pickle	Occasiona
76		*Clausena excavata*	Agnijhad	Ripe	Raw	Snacks	Occasiona
77		*Citrus medica*	Buru Nimbu(Kl)	Ripe/ matured	Raw and pickled	Juice, pickle	Frequent
78		*Limonia acidissima*	Kaintha(Ba)	Ripe	Raw, pickled	Chutney, pickle	Frequent
79		*Toddalia asiatica*	Baghranchuda(Kh)	Ripe	Raw, pickled	Pickle, flavour	Occasiona
80	Sapindaceae	*Schleichera oleosa*	Kusuma	Ripe	Raw/ boiled/pickled	Snacks	Frequent
81	Sapotaceae	*Xantolis tomentosa*	Kanta baula(San)	Matured	Cooked	Curry	Occasiona
82		*Madhuca indica*	Matkam(Kl), Mahula(Ba)	Ripe, unripe	Raw, cooked	Curry/ relishes	Frequent
83	Solanaceae	*Solanum virginianum*	Kanta Bheji(Kl)	Unripe	Fried/roasted	Curry	Occasiona
84		*Solanum torvum*	Denga Bheji(Kl)	Unripe	Cooked, fried	Curry	Normal
85	Sterculiaceae	*Guazuma ulmifolia*	Bursu (Kl)	Ripe	Raw	Snacks	Occasiona
86		*Sterculia urens*	Genduli(O)	Matured seed	Burnt	Snacks/ relishes	Occasiona
87	Tiliaceae	*Grewia subinequalis*	Pharsa (Kl)	Ripe	Raw	Dessert	Occasiona
88		*Grewia sapida*	Burso(Kl)	Ripe	Raw	Relishes	Normal
89	Ulmaceae	*Holoptelia integrifolia*	Charla(San), Jharanj (Kl)	Matured seed	Raw	Relishes	Normal
90	Verbenaceae	*Lantana camara*	Putusu (Kl)	Ripe	Raw	Snacks	Occasional
91		*Premna latifolia*	Gandhana(Kh)	Ripe	Raw	Snacks	Occasional
92	Vitaceae	*Ampelocissus latifolia*	Ie-Olan (Kl) Pechi (Kh)	Ripe	Raw	Snacks	Occasiona

Use category

The wild fruit plants grow naturally in the forest and are sources of food in the form of berries, seeds and nuts. This group of plants also fulfils needs of mankind such as medicine and other necessities. Species like *Aegle marmelos, Phyllanthus emblica, Cassia fistula, Capparis zeylanica, Clausena excavata, Litsea glutinosa, Solanum viginianum, Scindapsus officinalis* etc have more than one use in form of medicinal aspect.

Consumption pattern

The wild fruits are consumed in varieties of ways and are prepared using diverse recipes in accordance with local traditional knowledge and practice. Some of them are eaten raw, whereas others are eaten cooked and/ or processed. However, few of them are used in preservative form involve pickles, juice, candies and sauces. Some wild fruits are also used for preparation of sherbets, cooling drinks and distilled alcoholic beverages. Four traditional techniques such as eating raw, cooked/ boiled, roasted and pickled were observed for consumption of wild fruits. The raw fruits are mostly consumed ripen. The species consumed in raw form is 64, followed by cooked/boiled 31, pickled 10 and burnt 7 only (Table 1).

Many of the wild fruits are eaten raw just after collection and further peeling the outer skin for its good taste, while some of the wild fruits were eaten boiled/cooked and consumed during the food scarcity or lean periods. The species such as *Artocarpus lacucha, Butea superb, Capparis zeylanica, Catuneragam spinosa, Garcinia xanthochymus, Dillenia pentagyna, Scindapsus officinalis, Solanum torvum* and *Tamilnadia uliginosa* were successively boiled and taken as vegetable or during the lean periods. The Kolha and Khadia tribes of Similipal consume the unripe fruits of *Thladiantha cordifolia,* a wild bitter gourd (*Buru kirla*), as vegetable along with day meal (Misra and Sahoo, 2014). Now a day's many of the tribal people have shifted to foot hill region and adjoining plain areas and influenced by modern dietary trends and accordingly added different form of sweets, spices, oil, chilly and sour to enhance taste of cooked/ boiled items. Some fruits were reported to be eaten burnt as such after washing and removing the skin. In this method, the fruit or seeds are burnt whole or part or roasted not only to facilitate in removing the outer hard epicarp but also to enhance the taste of the item and to remove the anti-nutritional factors. Few species such as seeds of *Bauhinia vahlii, Gnetum ula, Cassia fistula* and fruit of *Aegle marmelos* were roasted/ burnt in the fire or embers and consumed as snacks which were preferred much by children. Some of the fruit yielding species found in wild have been domesticated by the local tribes due to their wider use as food. These are grown in backyards, homesteads, kitchen gardens or in fields using intensive farming that result in fruit yields to supplement the domestic use or as source of household income. These species are *Aegle marmelos, Sygygium cumini, Dillenia indica, Mangifera indica, Artocarpus lacucha, Spondias pinnata, Ziziphus* spp., *Schlechera oleosa, Madhuca indica* etc.

Income generating species

Some wild fruit species are marketable and it provides good opportunity for making additional income by sale/ trade in local markets. The tribes of the region practise to collect a number of fruits and sell them in local weekly markets or small towns in lieu of sash, cloth, salt or other commodities for their subsistence. The list of 18 major traded species was given in Table 2. Selling of fruits brings minimum return due to fairly low keeping quality and market costs. Therefore, some value addition process in form of preparation of pickles, chutney, jam, jelly, etc. may increase fruit shelf-life and economic profit to the local communities. This reflects a clear need to diversify the product base and to ensure that wild edible plants fetch higher prices per unit weight of produce for long-term interest of the people. Efforts have also been made to collect the data on wild edible fruits regarding some species preferred by children of age group between 6 and14 years. Out of 92 species, 68 species were commonly preferred by children.

Table 2. List of traded wild edible fruits

Sl. no.	Species of indigenous fruits on trade	Rate (₹ kg^{-1}) at local markets
1	*Aegle marmelos*	40-50
2	*Artocarpus lacucha*	20-30
3	*Bauhinia vahlii* (seeds)	40-50
4	*Buchanania lanzan* (kernel)	100-200
5	*Diospyros embryopteris*	20-30
6	*Diospyros melanoxylon*	15–17
7	*Madhuca indica*	22-30
8	*Mangifera indica*	20–25
9	*Momordica dioica*	60-80
10	*Phoenix acaulis*	10–12
11	*Phoenix sylvestre*	30-40
12	*Protium serratum*	40-50
13	*Schleichera oleosa*	15-20
14	*Semecarpus anacardium*	30-40
15	*Shorea robusta*	12-15
16	*Spondias pinnata*	20-30
17	*Syzygium cuminii*	40-50
18	*Ziziphus mauritiana*	30-40

CONCLUSION

The systematic survey, collection and documentation of information on indigenous traditional knowledge of wild edible fruit plants revealed that these neglected/ underutilised species have a great economic potential for sustainable livelihood of local inhabitants of Similipal Biosphere Reserve. This ethno-botanical information could significantly contribute to our knowledge tracing out the sources of edible plants to cater the needs of the human beings. An organised collection mission from diversity rich areas and characterisation/ evaluation of germplasm of the underutilised and agri-horticultural important fruit species are urgently required in view of the loss of genetic resources and erosion of traditional knowledge associated with these species. It was also observed that high market demand of fruits of few several species such as *Aegle marmelos*, *Artocarpus lacucha, Baccaurea ramiflora, Buchanania lanzan, Spondias pinnata, Citrus medica, Phyllanthus emblica, Diospyros melanoxylon, Mangifera indica, Protium serratum, Capparis zeylanica, Garcinia cowa, Cycas circinalis, Dillenia aurea, Limonia acidissima and Ziziphus mauritiana* coupled with no organised practice of extraction/ harvest caused a maximum economic loss. Further, they sell to the middlemen at a very low cost; therefore, organised efforts should be made to fulfil this objective. Optimum domestication of these wild fruit plants will not only improve the economic condition of the local communities but also help in the conservation of biodiversity for future use. Besides, few species such as *Baccaurea ramiflora, Garcinia cowa, Rubus ellipticus, Gardenia gummifera* and few others have severely declined their population and are found as rare/ endangered and in isolated habitats. Thus, public awareness and community based programmes need to be encouraged at all levels for *in-situ* and *ex-situ* conservation, efforts for value addition and large scale cultivation of species of future potential. Further, investigation on bio-chemical analysis of less known wild fruits may prove their suitability for selection of superior types for cultivation which may lead to the economic gain of the country. Adoption of these recommended species in traditional agro-forestry system and horticultural support programmes should find a place in the ongoing agricultural development programme of the country.

ACKNOWLEDGEMENT

Authors are grateful to the Similipal Biosphere Reserve Authority, Baripada, Department of Forests and Environment, Government of Odisha and Ministry of Environment and Forests, Government of India for providing financial assistance to the research project and permission of entry to the Biosphere Reserve. The Chief Executive, Regional Plant Resource Centre, Bhubaneswar is gratefully acknowledged for

providing support and facilities during the research study. The authors are also thankful to the tribal informants who shared valuable information on the traditional knowledge during the course of investigation.

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e-planet **14** (2): 57-64 (December 2016)

Dietary incorporation of *Allium sativum* on liver enzymatic activity of rohu, *Labeo rohita* (Hamilton, 1822) fingerlings

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Date of receipt : 15. 08. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

Rohu (*Labeo rohita)* is the most popular species cultivated in Indian sub-continent since it is highly delicious and most preferable carp among other Indian carps. Present study was carried out at wet lab of Fish Health Management Division, ICAR-CIFA, Bhubaneswar. After 30 days acclimatizing, *Labeo rohita* fingerlings (10 ± 2 g) were fed on diets supplemented with *Allium sativum* powder in duplicate containing 30 fishes at the rate of 0, 1, 5 and 10 g kg^{-1} for a period of 60 days. Serum samples were collected at 20 days interval and assayed for enzymatic parameters *viz.*, serum aspartate amino-transferase (AST), serum alanine amino-transferase (ALT) and alkaline phosphatase (ALP). After 60 days feeding, fishes were challenged with a pathogenic strain of *Aeromonas hydrophila* and mortalities were recorded over 10 days post-challenge. Serum AST and ALT activities were significantly ($p \leq 0.05$) different in all the treated groups of fish on day 20 and on day 10 bacterial post-challenge as compared to control. The highest survival on 10^{th} day post-challenge (85%) was recorded in group fed with 0.1% and 0.5% garlic supplemented diets. The intake of *A. sativum* at different dosages altered the AST, ALT and ALP activity but the variation was within the normal ranges that did not hamper the growth of fish. The study indicated that dietary supplementation of *A. sativum* did not negatively affect liver function and was found to have growth promoting activity in rohu.

Keywords: *Aeromonas hydrophila*, *Allium sativum*, *Labeo rohita,* liver enzymatic parameters

INTRODUCTION

Aquaculture in Asia has established itself as an important farming sector contributing 50% of the global food fish consumption (De Silva and Davy, 2010). During the last two decades, Indian aquaculture has registered over six fold growth where fresh water aquaculture contributed over 95 per cent of the total aquaculture production. Fish production in India has increased from 4.16 million tonnes in 1991-92 to 9.07 million tonnes in 2012-13 in which Indian major carp comprising family cyprinids contributed the bulk of fresh water production with over 1.8 million tonnes (FAO, 2014). Among cyprinids, *Labeo rohita* (Rohu) is the most popular species cultivated in Indian sub-continent. Rohu is highly delicious and most preferable carp among other Indian carps (FAO, 2001). The increase in aquaculture is said to be paralleled with a corresponding increase in the occurrence of infectious diseases, resulting often from high stocking densities and stress conditions that favor

the occurrence and spread of pathogens(Li et al., 2004). Cultured fish suffer a wide variety of bacterial, viral, parasitic and fungal diseases (Yunxia et al., 2001). At many fish farms and hatcheries several antibiotics, vaccines and chemotherapeutic agents as well as some immunostimulants have been used to prevent these diseases.

However, some of the immuno-stimulants cannot be used because of various disadvantages such as high cost and limited effectiveness. Besides, a large number of plants have been used in traditional medicine for the treatment and control of several diseases (Duke, 1987).

Garlic is a medicinal plant rich in calcium, phosphorus and vitamin B1. It also contains iodine salts which is helpful on cardio-vascular system and rheumatism, silicates are helpful on skeletal and circulatory system and sulfur salts help on the skeletal system, cholesterolemia and liver diseases. Garlic also contains carbohydrate, vitamin B complex, vitamins A and C (Dragan et al., 2008).

Garlic has shown antimicrobial (Kumar and Berwal, 1998), antifungal (Fromthing and Bulmer, 1978), antiviral (Harris et al., 2001), insecticidal (Wang et al., 1998), antihypertensive (Suetsuna, 1998), hepato-protective (Wang et al., 1998), inhibit tumor metabolism (Sumiyoshi, 1997), immuno-stimulant (Lewis and Elvin-Lewis, 2003) and immune enhancing activities (Sumiyoshi, 1997, Kyo et al., 1998). Garlic extract has also been shown to reduce serum cholesterol levels (Bordia et al., 1975, Augusti, 1977) and increase blood coagulation time (Bordia et al., 1975).

Garlic has got antibacterial and antifungal activity in animals including fish (Adetumbi et al., 1986, Rees et al., 1993 and Corzo-Martinez et al., 2007). Garlic supplementation in feeds has also been reported to increase growth performance in fish (Metwally, 2009). According to Sheela and Augusti (1992) and Diab et al. (2002) garlic has the ability of enhancing catalase activity in serum and lowering the levels of plasma glucose in fish. The non-specific defence system of *Oreochromisniloticus* has been improved by the inclusion of garlic in fish feed (Diab et al., 2002, Sahu et al., 2008). Garlic as a natural product and an immuno-stimulant may be useful in combating diseases in *Clarias gariepinus* which is one of the commonly farmed catfishes in Nigeria due to its ability to tolerate adverse environmental conditions (Holden and Reeds, 1972).

The present study evaluated the effect of dietary *A. sativum* on activity of specific liver enzymes *viz*., aspartate amino transferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in rohu *Labeo rohita* fingerlings.

MATERIALS AND METHODS

Experimental fishes

Fingerlings of rohu, *Labeo rohita* (average wet weight 10 ± 2 g), collected from the ICAR-Central Institute of Freshwater Aquaculture fish farm, were stocked in a 500 litre tank and kept for quarantine and health check. After quarantine, fish were acclimatized for 30 days in 400 litre chlorine free tap water and fed with commercial diet. Water exchange (30%) was done daily and water quality was monitored throughout the experiment at weekly intervals. The physico-chemical parameters e.g. temperature (28 ± 1^{o}C); dissolved oxygen concentration (6.0 ± 0.4 mg l^{-1}); ammonia-nitrogen concentration (0.14 ± 0.08 mg l^{-1}) and nitrite-nitrogen (0.04 ± 0.03 mg l^{-1}) were recorded during the experimental period. Fish were fed with their respective diet at the rate of 4% of body weight per day throughout the experiment (Table 1). The daily ration was subdivided into two and fed at 09.00 hours and 17.00 hours.

Garlic

One kg of garlic (*Allium sativum* L.) bulbs was collected from the local market and oven-dried at 60^{o}C, powdered by mortar and pestle and sieved. For each experiment, the required percentage (0.1%, 0.5%, 1.0% dry weight basis) was included in the feed. These represent diets B, C and D, respectively. Diet A (no garlic) served as control.

Preparation of herbal diets

Proximate composition of the basal diet was 39.6% crude protein, 7.2% lipids, 14.6 % ash, 7.1% moisture and 3% fibre (Table 1). Three test diets were prepared by incorporating garlic powder at concentrations of 1 g, 5 g and 10 g kg^{-1} feed. Dry ingredients were mixed thoroughly and 1% binder was added. Water was added and mixed thoroughly in a mixer for 20 min. The resulting dough was pelleted, dried at room temperature for 48 h and then stored in airtight containers until fed.

Experimental design and feeding diet

Rohu fingerlings (n = 240) were selected for the study and divided into 4 groups (A, B, C and D). Each group of 60 fingerlings was again divided into two equal duplicate subgroups. Group A was fed with basal diet and acted as the control. The remaining groups were fed with 1 g garlic kg^{-1} of feed (Group B), 5 g garlic kg^{-1} (Group C) and 10 g garlic kg^{-1} of feed (Group D) for 60 days. Blood and serum samples were collected from fish in each subgroup and examined for the following liver enzymatic parameters *viz.* serum aspartate amino-transferase(AST), serum alanine amino-transferase (ALT) and alkaline phosphatase (ALP).

Pathogens

Aeromonas hydrophila (ATCC 49040) maintained in Fish Health Management Division, ICAR-CIFA was used in this study. They were cultured in nutrient broth (Himedia) for 24 h at 37°C. The broth culture was centrifuged at 3000 rpm for 10 min. The supernatants were discarded and the pellets were resuspended in phosphate-buffered saline (PBS 7.4), and the optical density of the solution was adjusted to 0.5 at 456 nm, which corresponded to $1x10^7$ cells ml^{-1}.

Collection of blood

Feed was withheld from fish for 24 h before blood samples were collected. From randomly picked fish (n = 20 from each sub-group) at 20-day intervals, after anaesthetizing with 0.1 ppm MS-222, blood was collected from the caudal vein with a 1 ml plastic syringe ringed with heparin and stored at 4°C and used the same day. Blood samples were also collected without heparin, allowed to clot, centrifuged at 7000rpm and sera collected and refrigerated. From each subgroup 12 and eight fish were sampled for serum and blood, respectively, and returned to their respective system.

Sera were pooled into four groups, depending upon volume, for estimation of enzymatic parameters.

Enzymatic parameters

AST assay

Serum AST was determined following Wallnofer et al. (1974). Serum (200 ml) was added to 2 ml of the test reagent, mixed thoroughly and incubated for 1 min at 37°C. Subsequently, 200 ml of α-oxoglutarate was added. The solution was mixed thoroughly and the initial absorbance was recorded at 340 nm. Three subsequent readings were taken at 1 min intervals. AST activity was expressed as U l^{-1} of serum. The final concentration was calculated as:

AST activity (U l^{-1})= 1905 x Δ A 340 nm min^{-1}

where 1U = 16.67 x 10^{-3} μ kat

ALT assay

For estimation of serum ALT (Wallnofer et al., 1974) serum (200 ml) was added to 2 ml of test reagent, mixed thoroughly and incubated at 25°C for 1 min. Subsequently, 200 ml of α-oxoglutarate was added and the solution was mixed thoroughly. Initial absorbance was recorded at 340 nm. Three subsequent readings were taken at 1 min intervals as above. The ALT activity was expressed as U $l^{-1}$of serum. The final concentration was calculated as:

ALT Activity (U l^{-1})=1905 x Δ A at 340 nm min^{-1}

where 1U=16.67 x 10^{-3} μ kat

ALP assay

Serum ALP was determined as per Rosalki et al. (1993). Serum (50 ml) was added to 3 ml of test reagent containing diethanolamine buffer, Mg

Cl_2 and p-nitrophenyl phosphate. This was mixed thoroughly and the initial absorbance was recorded at 405 nm. Three subsequent readings were taken at 1 min intervals at 405 nm. ALP activity was expressed as U $l^{-1}$of serum. The final concentration was calculated as:

ALP Activity (U l^{-1})

= 3300 x ΔAsample at 405 nm min l^{-1}

1U = 16.67 x 10^{-3} μ kat

where ΔA = change in Absorbance per min

Challenge study

After 60 days of feeding, the experimental fish were challenged with *Aeromonas hydrophila* (ATCC 49040). These bacterial suspensions having $1x10^7$ cells ml^{-1} were serially diluted using standard dilution technique with PBS and used for the challenge. Each fishes were challenged with 100 μ l of bacterial suspension, which corresponded to 10^5 cells ml^{-1}. At the end of 10 days of challenge, sera samples of different challenged groups were collected for enzymatic parameters.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and mean differences among experimental groups were evaluated using Duncan's multiple range tests (DMRT) (Duncan, 1955) at $p< 0.05$ significance level.

RESULTS AND DISCUSSION

AST activity was significantly ($p \leq 0.05$) different in the group of fish (Group C and Group D) at 20th day and after bacterial post challenge and group D at 40th day of exposure period as compared to the control (Table 2). However, the AST activities were decreased in all the treatment groups from day 20 to day 60 and after challenge as compared to the control groups. There was a decrease ($p> 0.05$) in ALT activities in all the treatment groups over entire assay period as compared to the control except Group B and C on day 40, Group B on day 60 (Table 2). ALP activity was significantly ($p \leq 0.05$) different to the group of fish Group B at 20th day as compared to control. But there was insignificant ($p> 0.05$) difference of ALP level was found in all the treatment groups at 40th, 60th and after bacterial post challenge as compare to their respective control (Table 2). In Group B and C groups ALP activities were increased from day 20 to day 60 and decreased after challenge but in Group D, it was increased upto day 40 and thereby decreased.

Liver specific enzymes such as ALT and AST are more sensitive measures of hepatotoxicity and can be measured in a short period (Balint et al., 1997). But, Oluah (1999) noted that changes in the ALT and AST values showed due to tissue damage in different organs like liver, kidney, muscle and gills. ALT and AST are indicators of liver function (Ozaki, 1978) and damage (Oda, 1990). Pradhan (2012) noticed that dietary stimulation increasing the serum/blood immune parameters and liver enzyme activities thereby enhances the resistance against infection. The ALT activity was found to decrease in all groups of fish fed garlic supplemented diets in comparison to control group, over all the experimental days feeding and in pathogen challenged groups, indicating that the liver function was not impaired due to garlic feeding.

In our present study, it was found that AST activity decreased significantly ($p< 0.05$) in group D fed with garlic at day 20, 40 and on day 10 post challenge. Shalaby et al. (2006) showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol. Similar type of observations were reported by El-Shater et al. (1997) and Augusti et al. (2001), who found that the lipid parameters and enzyme activities (AST, ALT, and ALP) in rat serum decreased significantly when they were fed a diet containing 5 % *Allium sativum*. Ajeel and Al-Faragi (2013) recommend use garlic at 10g kg^{-1} in feed of common carp reduced ALT and AST activity that may cause stabilized cell membrane, protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. The reduction of liver enzymes helped the liver to

maintain its normal function by accelerating the regenerative capacity of its cells. Sahu et al. (2008) reported fed with turmeric incorporated diet to rohu fingerlings for a period of 60 days decreased serum AST and ALT activities and opined that glutamate and oxaloacetate were unavailable to the Kreb's cycle through this transmission route. AST usually found in the liver, muscle, kidney, brain, lungs, cardiac, pancreas, leucocytes and erythrocytes whereas ALT is present in liver in highest concentration (Kupeli et al., 2006).

Al-Salahy and Mahmoud (2003) studied orally administered 2g kg^{-1} body wt. of garlic (*Allium sativum*) juice on *Chrysichthys auratus* and showed insignificant changes in both serum AST and AST in spite of the degeneration of some hepatocytes seen in histological observation. Such results suggest an inhibitory effect of raw garlic juice on the activity of liver ALT (a more specific enzyme to the liver) and AST. Also Unnikerishnan et al. (1990) and Hattori et al. (2001) found same result of decreases serum ALT and AST by incorporation garlic at different doses in mammals. Moreover, assay of plasma ALT activity, an indicator of liver necrosis, showed that dialy disulphide isolated from garlic treatment (200 mg/ kg body wt), effectively protected the liver of mice against acetaminophen (Zhao and Shichi, 1998). Ajoene (20-100 mg kg^{-1} body wt) derived from garlic suppressed the rise in serum ALT activity in acetaminophen-induced liver injury in mice (Hattori et al., 2001).

However, a high dose of garlic (2 ml garlic oil per 100 g body wt.) led to significant rise in serum AST in rats (Joseph et al.,1989). In the present work, it could be suggested that the damaged effect of garlic on liver did not reach such levels as to cause elevations of serum transaminases. Banerjee et al. (2001) found that lower doses of garlic have the potential to enhance the endogenous antioxidant status, while at higher doses, the reverse of this effect is observed. Sumiyoshi et al. (1984) found dietary intake of garlic extract at 2g kg^{-1}/ 5 times/week for 5 months did not show any toxicity symptoms in rats. However, a low dose of garlic (50 mg/ kg body wt daily for 14 days) reduced ALT in mice treated with a chronic lethal dose of cyclophosphamide (Unnikerishnan et al. 1990).

Phosphatase activity is of significance in pathological conditions (Reddy and Rao, 1990). In our present study, insignificant increased ($p > 0.05$) ALP activity was found in the group of fish fed with garlic over different days except 30 days of feeding and at 10 day post-challenge in groups B and D. Increased ALP activity was reported by Sahu et al. (2008) while feeding rohu at various doses of turmeric for a period of 60 days following challenge with *A. hydrophila*. Also, serum ALP showed a rise only in the case of extrahepatic obstruction, intrahepatic cholestasis, infiltrative liver disease and hepatitis (McIntyre and Rosalki, 1991).

Increase in phosphatase activity indicates higher breakdown of the energy reserves, which is utilized for the growth and survival of fish. ALP is the brush border enzyme, which splits various phosphorous esterase at an alkaline pH and mediates membrane transport (Goldfisher et al., 1964). ALP is also involved in transport of glycogen (Guptaand Rao, 1974), protein synthesis (Pilo et al., 1972) and synthesis of certain enzymes and secretary activity. Thus, any alteration in the activity of ALP may affect an animal in a variety of ways.

The effect of garlic on the *Labeo rohita* in biochemical and immunological parameter has been reported earlier (Sahu et al., 2007). According to our previous finding, it was noticed that feeding with 0.1%, 0.5% and 1.0% garlic increase the survivability after post challenge with *Aeromonas hydrophila* upto 85.71% and 71.42% respectively. The present study revealed that there was alteration of liver enzyme which was lower as compared to the control group indicating garlic fed diet could not make the fish under stress. Thus it could be concluded that feeding at 1.0 % level could enhance the survivability without impairing liver function.

Table 1. Per cent inclusion of the ingredients in experimental diets with desired crude protein and lipid level

Ingredients	Per cent inclusion
Groundnut oil cake	40
Fish meal	25
Rice bran	20
Soybean meal	12
Vitamins *and* Minerals mixture	2
Starch	1
Calculated crude protein (%)	40
Calculated lipid (%)	5

Table 2. Effect of oral feeding of garlic powder on enzymatic parameters of *L. rohita* followed by i. p. challenge of *A. hydrophila* after 60 days

			Before challenge		After challenge
Parameter	Group	20 days	40 days	60 days	70 days
AST	GC	40.00 ± 3.29 ^{a}	38.73 ± 4.57 ^{a}	25.3 ± 3.36 ^{a}	32.82 ± 1.62 ^{a}
(u l^{-1})	G1	36.19 ± 2.90 ^{a}	33.65± 2.76^{ab}	24.13± 2.29 ^{a}	32.38± 3.96^{ab}
	G2	25.39 ± 2.76 ^{b}	32.38± 2.19^{ab}	22.85± 3.96 ^{a}	26.66 ± 2.91^{bc}
	G3	22.22 ± 2.76 ^{b}	23.49 ± 2.76 ^{b}	21.59± 3.36 ^{a}	21.58 ± 2.28 ^{c}
ALT (u l^{-1})	GC	29.20 ± 2.29 ^{a}	32.38± 2.91^{ab}	30.47± 1.56 ^{a}	41.78 ± 1.67 ^{a}
	G1	27.30 ± 1.68 ^{a}	43.81 ± 4.79 ^{a}	31.11± 2.41 ^{a}	33.9 ± 2.78 ^{b}
	G2	26.42 ± 2.41 ^{a}	43.18 ± 3.36 ^{a}	27.93± 2.13 ^{a}	28.57 ± 1.09^{bc}
	G3	24.76 ± 1.09 ^{a}	23.46± 3.35 ^{b}	22.82± 1.20 ^{a}	22.86 ± 2.19 ^{c}
ALP	GC	24.48 ± 0.68 ^{a}	34.08 ± 2.19 ^{a}	36.52± 3.16 ^{a}	27.29 ± 1.82 ^{a}
(u l^{-1})	G1	16.4 ± 1.05 ^{b}	37.73 ± 4.12 ^{a}	33.81± 4.09 ^{a}	28.51 ± 3.21 ^{a}
	G2	25.55 ± 1.82 ^{a}	33.82 ± 1.36 ^{a}	34.72± 1.38 ^{a}	31.85 ± 3.29 ^{a}
	G3	29.82 ± 2.25 ^{a}	31.03 ± 2.15 ^{a}	29.58± 2.34 ^{a}	27.80 ± 1.90 ^{a}

Note: Data are expressed as mean ± S.E. Superscript column wise on right hand side for particular treatment group are significantly ($p < 0.05$) different from the control group

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e-planet **14** (2): 65-69 (December 2016)

Seroprevalence of Bluetongue among goat population of Odisha

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Date of receipt - 14. 07. 2016 Date of acceptance- 22. 11. 2016

ABSTRACT

Serum-analysis was performed during October 2015 to April 2016 to ascertain prevalence of Bluetongue (BT) virus infection among goat population in Odisha. Samples were collected randomly from apparently healthy goats of all the 30 districts of Odisha encompassing 10 different agro-climatic zones. Anti-BT antibodies were screened in sera using indirect enzyme linked immunosorbent assay (i-ELISA) at Division of Virology, Indian Veterinary Research Institute, Mukteswar, Uttar Pradesh. Out of 289 samples screened, 53.63% samples were found positive for Bluetongue virus infection. The prevalence of anti-BT antibodies in different agro-climatic zones ranged between 25.0 to 66.0%. None of the samples collected from Boudh district were found positive to BT infections. Contrary, all the samples of Jagatsinghpur districts were found to be positive. This seroprevalence picture of bluetongue, first of its kind, unfolds this viral infection in Odisha.

Key words: Seroprevalence, bluetongue, i-ELISA, goat, Odisha

INTRODUCTION

Bluetongue (BT), an important viral disease of small ruminants, is enlisted in list A arthropod-borne haemorrhagic viral disease by OIE (Breard et al., 2004). Historically, the name 'bluetongue' (blue tongue, blue-tongue) is the English meaning of the Afrikaans word 'Bloutong', which was coined by Boer farmers just in a way to describe the distinctive cyanotic tongue of some severely affected sheep by the virus. Subsequent to its first report from South Africa wayback 1902 as fever/ malarial catarrhal fever/epizootic catarrh of sheep, it is now identified across all continents except Antarctica (Maclachlan and Guthrie, 2010). Though the disease affects both domestic and wild ruminants of semi-tropical and temperate regions of the world ranging between the latitudes of 35^{o} south and 40^{o} north, it is mostly considered as a disease of sheep and goat causing remarkable economic losses in terms of morbidity and mortality.

In India, the disease was first reported during 1961 from Maharashtra causing a severe loss in sheep population (Sapre, 1964). Report says that 11 states of India are under the grip of BT either on the basis of viral isolation or by the detection of group specific antibodies (Prasad, 2000). Disease is characterised by mild febrile illness to extensive erosive lesions of oral mucosa with coronitis, oedema of the head and muzzle, stiffness of limbs

and oedema of head and neck leading to death. Field level clinical sign-based diagnosis is difficult due to overlapping of clinical manifestations; the disease is either misdiagnosed or under reported. Available literatures/reports indicate absence of active prevalence of BT in the eastern and north-eastern part of India (Prasad and Srivastava, 1995, Joardar et al., 2009). Non-availability of desired information provoked to undertake a systematic study on seroprevalence of bluetongue among goat population of Odisha to ascertain the ground reality.

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MATERIALS AND METHODS

Serum samples from goats were collected randomly between October-2015 to April-2016 covering10 agro-climatic zones of Odisha. 2 ml of blood samples were collected in clot activator vials from 289 goats of different categories. Concerned farmers and vets were simultaneously interrogated to collect the information suggestive of BT in their farm or locality, if any.

Procedure of serological assays using indirect-ELISA

The methodology described for detection of BT antibodies in serum is indirect ELISA based on VP-7 protein, which has been developed at Indian veterinary research institute (IVRI), Mukteswar (Chand et al., 2009). Diluted BT viral antigen (rVP7) of 50µl was added to coat each well of the plate and kept overnight at 4^{o}C. After blocking the uncoated portions of the wells with blocking buffer, 50µl of diluted known positive, known negative and test serum were put into the wells and kept in room temperature for 1 hour. After washing the plate three times, 50µl of diluted conjugate was added to all wells except the conjugate control and kept for 1 hour at room temperature. Then, 50µl of chromogen-substrate (ortho-phenylenediamine, OPD) solution was added to all wells. It was kept for 10min in dark till the colour develops and then 50µl of stop reagent (1M H_2SO_4) was given to all wells. Finally, reading was taken in an ELISA plate reader at 492 nm. The average optical density (O.D.) values of negative control is calculated and compared with the test O.D. values. The O.D. values of tests that were higher than the average O.D. values of the negative control were considered as positive for anti-BT antibodies.

The results of the investigation were subjected to Chi-square test using SPSS software (Indian version) to determine the difference in susceptibility with $p \leq 0.05$.

RESULTS AND DISCUSSION

Analysis of 289 serum samples from goats across the 10 agroclimatic zones of Odisha, 53.63% were positive for BT with 57.03% (77/135) in males and 50.64% (78/154) in females. Earlier study conducted between October 2011 and March 2012 from two different geographical pockets of Odisha e.g., coastal and central regions showed an overall prevalence rate of 52.43% in goat (Pany et al., 2016). The difference between sex recorded here was found to be at a non-significant label ($p > 0.05$). Of the total serum samples processed, the highest prevalence of 66.0% was found in East and South Eastern coastal plain (Puri, Cuttack, Khorda, Nayagarh, Jagatsinghpur and Kendrapada) and lowest of 25.0% in South Eastern Ghat (Malkangiri). District-wise data revealed that Jagatsinghpur district had highest (100%) prevalence of BTV antibodies whereas all the serum samples of Boudh were found negative for BTV

(Table 1). Odisha having diverse agroclimatic zones falls on tropics (17°49'- 22°36' N latitudes and 81°36' - 87°18' E longitudes) and likely to be endemic to Bluetongue virus. This hypothesis is proved in the present investigation. As there is statistically significant ($P< 0.05$) difference in seroprevalences it could be correlated to the variable herd immunity and other predisposing environmental factors that affect the label of virulence of BTV.

BTV has got its endemicity across many parts of the world with 26 distinct recognised serotypes (BTV-1, -2, -3, etc.), with no cross protectivity (Maan et al., 2012), against which, India has been reported with 21 serotypes prevalent across the country (Wilson et al., 2000 and Maan et al., 2011). With fact that BT is a disease of sheep, most of the BTV isolates in India are from goats suffering from Peste des Petits Ruminants (PPR) infection (Biswas et al., 2010) and the serotype-25 is more pathogenic to goats (Hofmann et al., 2008).

As reported earlier by Audarya et al. (2015), the overall sero-positivity among non-descript adult goats of Delhi were 13.21%. An overall prevalence rate of 7.5% among goats of Kozhikode district and 16% among sheep of Palakkad district of Northern Kerala was reported by Arun et al. (2014). Seropositivity of 58.82% among sheep and 31.79% among goat was reported by Joardar et al. (2013) in Assam. In the state of Uttar Pradesh, sero-positive among sheep and goat were found to be 28.6% against BT (Bitew et al., 2013). Out of 11 states of India, antibodies against the virus were detected from 8 states with a sero-positivity of 25.0% among the test samples from sheep and other domestic and wild animals (Mehrotra and Shukla, 1990).

Small ruminants in Odisha are predominately maintained as nomadic or free grazing lands. The higher prevalence rate recorded in the present investigation might be due to close contact with infected animals or migrated ones into the state. Odisha being a rich niche of wild fauna in free ranging, contribution of wild counterparts could be another potential source of infection. The movement of the vector, *culicoides* also play a major role in transmission of the disease, as India has been reported with 39 species of *culicoides* out of the 1400 species prevalent world-wide, (Sreenivasulu et al., 2004) to carry the virus. Further, poor reporting, misdiagnosis or under diagnosis cannot be ruled out due to overlapping of clinical signs of BT to that of foot and mouth disease (FMD), ovine rinderpest, contagious ecthyma, and hemonchosis akin (Elbers et al., 2008). High seroprevalence of BT recorded during the present investigation provide an impetus to think on the contribution of BT as possible major causes of the economic loss goat farming.

Virus concentrations in secretions and excretions of infected animals are minimal, making direct, indirect, or aerosol transmission unlikely. *Culicoides* biting midges are the only significant natural transmitters of the virus. Distribution of BTV parallels the spatial and temporal distribution of vector species and the temperatures at which BTV will replicate in vectors.

Control of vectors by insecticides or protection from vectors may lower the number of *Culicoides* bites and subsequently the risk of exposure to BTV infection. Hence, prophylactic immunization remains the most effective and practical control measure against bluetongue in endemic regions. However, no commercial vaccine is available in India. Attenuated and inactivated polyvalent and monovalent vaccines against BTV are commercially available in some countries.

Use of vaccines with different serotypes does not provide consistent cross-protection. Live attenuated vaccines cannot be used during *Culicoides* vector seasons, because these insects may transmit the vaccine virus(es) from vaccinated to non vaccinated animals which in turn may result in reassortment of genetic material and give rise to new viral strains.

A feasible solution to all these issues could be conducting extensive epidemiological studies coupled with immuinization of susceptible stock using the vaccine produced by local strains.

Table 1. Seroprevalence of Bluetongue in different agro-climatic zones/districts of Odisha

Sl. No.	Agro-Climatic zones	Districts	% of positive samples
1	North Western Plateau	Deogarh Sundergarh	47.36
2	North Central Plateau	Keonjhar Mayurbhanj	58.33
3	North Eastern Coastal Plain	Bhadrak Baleswar	48.14
4	East and South Eastern Coastal Plain	Puri Cuttack Khordha Nayagarh Jagatsinghpur Kendrapada	66.0
5	North Eastern Ghat	Rayagarh Kandhamal Ganjam Gajapati	57.89
6	Eastern Ghat High Land	Koraput Nabarangapur	47.61
7	South Eastern Ghat	Malkanagiri	25.0
8	Western Undulating Zone	Kalahandi Nuapara	56.52
9	Western Central Table Land	Bargarh Sambalpur Bolangir Sonepur Boudh Jharsuguda	43.75
10	Mid Central Table Land	Angul Dhenkanal	57.89

CONCLUSION

A total of 289 serum samples collected randomly from apparently healthy goats of all the 30 districts of Odisha under 10 different agro-climatic zones were screened for anti- bluetongue (BT) antibodies by indirect enzyme linked immunosorbent assay (i-ELISA) at Division of Virology, IVRI, Mukteswar during October 2015 to April 2016. Prevalence of anti-BT antibodies in different agro-climatic zones ranged between 25.0 to 66.0 % with an overall rate of 53.63%. None of the samples collected from Boudh district were found positive to BT infection as against 100% in Jagatsinghpur district.

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e-planet **14** (2): 70-77 (December 2016)

Isolation, characterization and antibiogram study of *Pasteurella multocida* isolated from cattle in Odisha, India

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Date of receipt: 18. 08. 2016 Date of acceptance: 07. 11. 2016

ABSTRACT

Haemorrhagic septicaemia (HS), caused by the Gram-negative bacterium, *Pasteurella multocida* serotype B:2, is an economically important disease responsible for high mortality and morbidity of bovines in countries of south or south-east Asia and Africa. The present study was undertaken in 178 cattle suspected of haemorrhagic septicaemia in Odisha resulting in 6 nos. of *Pasteurella multocida isolates*. These isolates were identified through standard procedures like morphological, cultural, biochemical and serological tests. The biochemical and molecular characterization were conducted to confirm the presence of the specific organism causing H.S. Molecular characterization of the isolates was done by PM-PCR and HSB-PCR. In capsular typing, all the isolates were found to be capsular, type B; thus confirming them to be Haemorrhagic Septicaemia cases. The antibiogram study of the *Pasteurella multocida* isolates was carried out through *in vitro* antibiotic sensitivity test in which cephalosporins, quinolone compounds and chloramphenicol occupied the first position followed by aminopenicillins, amynoglycosides and oxytetracycline. Streptomycin and furazolidone were found to be resistant against *Pasteurella multocida*.

Keywords: *Pasteurella multocida*, antibiogram, cattle, molecular characterization

INTRODUCTION

Haemorrhagic septicaemia (HS) is an economically important disease responsible for high mortality and morbidity of bovines in countries of South or Southeast Asia and Africa. In India, it ranks first among bacterial pathogens and accounts for more than 40-55% of mortality due to infectious diseases of bovines (Dutta et al., 1990) and causes an annual economic loss of 225 million rupees (Singh et al., 2008). The causative organism *Pasteurella multocida*, is a gram-negative, cocco-bacillary, non-motile, non spore-forming, capsulated, facultative anaerobe belonging to the family Pasteurellaceae. The bacteria have been classified into five capsular types (A, B, D, E and F) with each capsular type predominantly associated with a particular disease type. Heamorrhagic septicaemia is caused by capsular type B in Asian countries and type E in African countries, whereas serotypes A isolates have been recovered from HS like symptom cases. Because of the severity of the disease, diagnosis of the disease from clinical sample is of utmost importance. The diagnosis of the disease is done primarily basing on clinical signs, gross pathological lesions, morbidity and mortality patterns, whereas

confirmation is carried by isolation, biochemical and molecular characterization of the bacteria. Due to injudicious and indiscriminate use of antibiotics, *P. multocida* has developed resistance to commonly used chemotherapeutic agents. Keeping the economic importance of HS in view the present study was undertaken to isolate of *Pasteurella multocida* from HS suspected cases, its biochemical and molecular characterization along with antibiogram study.

MATERIALS AND METHODS

Clinical samples (n=178) consisting of 96 nasal swabs, 38 blood samples, 11 lungs tissue and 33 milk samples (from those animals affected with mastitis) were collected from suspected cases of HS and processed at Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, OUAT, Odisha for isolation of *P. multocida*. Blood smears were stained with Gram's method of staining and examined microscopically. Smears were made from lungs tissues and stained with Gram's method of staining and examined microscopically. Pure culture was obtained and characterized as per the methods described by Buxton and Fraser (1977) and Cruickshank et al. (1980). Pathogenecity test for each isolate of *Pasteurella multocida* was conducted in Swiss albino mice as per the procedure described by Gupta et al. (1977) with due approval from animal ethical committee. Heart blood from the dead mice was streaked on the blood agar (BA) plate for re-isolation of the organism in order to confirm the cause of death. The isolates were subjected to *in-vitro* sensitivity tests using the disc diffusion method as described by Bauer et al. (1966) for different antibiotics obtained from Himedia, Mumbai (Table 1). Rapid slide agglutination test for each strain of *Pasteurella multocida* was carried out using the hyper immune serum developed in rabbits against the reference strain of *P. multocida* (strain P_{52}).

P. multocida species specific PCR was performed as described by Townsend et al. (1998a) using the primer pair KMT1T7 –KMT1SP6. In brief, the PCR reaction was performed in a reaction mixture of 25 μl containing 1x PCR buffer (10 mM Tris –HCl, pH 9.0, 50 mM KCl), 1.5 mM Mg Cl_2, 0.25 μM of each primers, 0.2 mM concentrations of each dNTPs, 1 unit of Taq polymerase and 2.0 μl of template DNA. Amplification was performed in a thermal cycler as per the amplification regime of 30 cycles consisting 30 cycles of denaturation at 95ºC for 45 seconds, annealing at 55ºC for 45 seconds, extension at 72ºC for 45 seconds and a final extension of 72ºC for 6 minutes. For easy and specific PCR assay, single bacterial colony grown in blood agar plate was picked up with the help of a sterile pipette tip and mixed in PCR mixture for amplification. In order to confirm capsular type a multiplex PCR was performed using primers specific for serotype A, B, D, F and E by using primer sets in the same reaction mixture (Townsend et al., 2000). Similarly, to confirm HS causing isolates in a single reaction, a duplex PCR was performed by taking both the primer sets (KMT1SP6-KMT1T7 and KTSP61-KTT72) in the same reaction mixture to obtain both the products at a time in positive cases (Townsend et al., 1998a).

RESULTS AND DISCUSSION

Out of the 178 samples processed, *P. multocida* was isolated from six cases (Table 2). The isolates were finally identified and characterized on the basis of morphological, cultural, biochemical and serological tests (Table 2). The bacteria revealed characteristic non-haemolytic, smooth, grayish, glistening, transluscent and iridescent small colonies on blood agar plates. Inoculum in MHA plate revealed dew drop like colonies whereas no growth was observed in MLA plate. Small gram negative coccobacilli were observed on the smears prepared from the culture by Gram's staining method. Results of the biochemical test were show in Table 3. Few of the isolates ferment raffinose, arabinose, sorbitol and lactose where as all the isolates are negative for fermentation of dextrin, ramnose, inulin, malonate and dulcitol (Table 4). Similar morphological, cultural and biochemical characters of *P. multocida* strains have been reported previously observed (Buxton and Fraser, 1977; Bain et al., 1982; Dabo et al.,

Table 1: Showing different concentration of antibiotics used in the study

Sl. No.	Name of the antibiotics	Concentration /Disc	Sensitive		Moderate		Resistant	
			Nos	%	Nos	%	Nos	%
1.	Ceftriaxone (Ci)	30mcg	6	100	0	0	0	0
2.	Cefaclor (Cj)	30mcg	6	100	0	0	0	0
3.	Cefotaxime (Ce)	30mcg	6	100	0	0	0	0
4.	Cephalexin (Cp)	30mcg	6	100	0	0	0	0
5.	Cephadroxil (Cq)	30mcg	4	67	2	33	0	0
6.	Cefixime (Cfx)	5mcg	4	67	2	33	0	0
7.	Ceftazidime (Ca)	30mcg	4	67	1	17	1	17
8.	Cefuroxime (Cu)	30mcg	4	67	2	33	0	0
9.	Moxifloxacin (Mo)	5mcg	5	83	1	17	0	0
10.	Levofloxacin (Le)	30mcg	4	66	1	17	2	17
11.	Ciprofloxacin (Cf)	30mcg	5	83	0	0	1	17
12.	Enrofloxacin (Ex)	10mcg	5	83	1	17	0	0
13.	Pefloxacin (Pf)	5mcg	4	67	1	17	1	16
14.	Ofloxacin (Of)	5mcg	4	66	1	17	1	17
15.	Sparfloxacin (S)	5mcg	4	67	2	34	0	0
16.	Ampicillin (A)	25mcg	3	50	2	34	1	16
17.	Amoxycillin (Am)	30mcg	4	66	1	17	1	17
18.	Cloxacillin (Cx)	10mcg	3	50	3	50	0	0
19.	Neomycin (N)	30mcg	2	34	2	33	2	33
20.	Tobramycin (Tb)	30mcg	3	50	1	17	2	33
21.	Gentamicin (G)	10mcg	3	50	2	33	1	17
22.	Amikacin (Ak)	30mcg	3	50	2	33	1	17
23.	Oxytetracycline (O)	30mcg	4	67	2	33	0	0
24.	Chloramphenicol (C)	30mcg	5	83	1	17	0	0
25.	Streptomycin (S)	25mcg	0	0	2	33	4	67
26.	Furazolidone (Fr)	50mcg	0	0	2	33	4	67
27.	Carbenicillin (Cb)	30mcg	3	50	3	50	0	0
28.	Netillin (Nt)	30mcg	0	0	2	33	4	67
29.	Cotrimoxazole (Co)	1.25/23.75mcg	2	33	1	17	3	50
30.	Erythromycin (E)	30mcg	3	50	2	33	1	17
31.	Roxithromycin (Ro)	30mcg	2	33	2	33	2	33
32.	Pipracillin / Tazobactum (Pt)	100mcg	6	100	0	0	0	0
33.	Azithromycin (At)	30mcg	3	50	1	17	2	33
34.	Ticarcillin/Clavulanic acid (Tc)	75/10mcg	5	83	1	16	0	0

Table 2: Isolation of *Pasteurella multocida* from different samples

Nature of Sample	Species	Respiratory infection			Suspected HS cases			Mastitis Cases		
		No. of Sample processed	No. of Isolates	%of Recovery	No. of Sample processed	No. of Isolates	%of Recovery	Sample processed	No. of Isolates	% of Recovery
Nasal swab	Cattle	96	1	1.04	28	1	3.57	-	-	-
Blood	Cattle	38	2	5.26	28	2	7.14	-	-	-
Lungs tissue	Cattle	11	3	2.81	11	3	27.28	-	-	-
Milk	Cattle	-	-	-	-	-	-	33	Nil	-

Table 3: Results of biochemical reactions of *Pasteurella multocida* isolates

Sl.No.	Biochemical Tests	Isolates					
		1	2	3	4	5	6
1.	Indole	+	+	+	+	+	+
2.	Catalase	+	+	+	+	+	+
3.	Oxidase	+	+	+	+	+	+
4.	Nitrate reduction	+	+	+	+	+	+
5.	Citrate utilization	-	-	-	-	-	-
6.	H_2S Production	+	+	+	+	+	+
7.	Gelatin liquefaction	-	-	-	-	-	-
8.	Urease	-	-	-	-	-	-
9.	M.R	-	-	-	-	-	-
10.	V.P.	-	-	-	-	-	-
11.	Lysine decarboxylase	-	-	-	-	-	-
12.	Ornithine decarboxylase	+	+	+	+	+	+
13.	Esculin hydrolysis	-	-	-	-	-	-
14.	Phenylalanine	-	-	-	-	-	-
15.	β-Galactosidases	-	-	-	-	-	-
16.	β-Glocuronidases	+	+	+	+	+	+
17.	N acetyl Glucosamine	+	+	+	+	+	+

2007; Kumar et al., 2009). All the isolates were found pathogenic to mice by intraperitoneal route and the mice died within 24 hour post-inoculation. Petechiae in the pericardium, congestion of lung, liver and spleen were observed as gross lesions in mice, which died after experimental infection. Bipolar stained organisms could be demonstrated in the heart blood and impression smears of spleen and liver by Gram's staining. The organism was also isolated from heart blood and visceral organs. Similar observations have been previously observed by Shivashankara et al. (2000) in bovine isolates. The isolates were also confirmed as *Pasteurella multocida* by rapid slide agglutination test using

the hyper immune serum developed in rabbits against the reference strain of *Pasteurella multocida* (strain P_{52}). All the isolates showed appearance of coarse flocullar agglutination within 30 seconds indicating positive reaction and the index for agglutination is 1 for *P. multocida*. Rapid slide agglutination test for confirmation of *P. multocida* has been previously reported by Sheikh et al., 1995.

Six isolates of *Pasteurella multocida* were recovered altogether from 178 samples, the percentage of which was only 3.37 per cent indicating that the incidence of haemorrhagic septicaemia in Orissa to be low which might be due to regular preventive vaccination policy practiced in the state. Out of 60 nasal swabs from respiratory infected animals and 28 nasal swabs from suspected HS cases, only one isolate could be recovered which suggests that the organism are not consistently present in nasal secretions of sick animals (De-Alwis, 1989). Blood samples from suspected Haemorrhagic Septicaemia cases yielded two isolates which clearly indicate that timely collection of blood samples with apparently clinical symptoms is the crucial factor for recovery of *P. multocida*.

Isolation of *P. multocida* from eleven lungs tissue samples from animals showing pneumonic changes revealed three isolates. The low recovery rate in the given study attributes to the association of various other organisms like Streptococcus, Mycoplasma, Haemophylus, Para Influenza 3, Infectious Bovine Rhino-tracheitis, Bovine respiratory syncitial virus, Bovine Adenovirus and Rhinovirus etc. which might be responsible for causation of pneumonia in cattle and buffaloes (Vogel et al., 2001)

Table 4: Results of sugar fermentation test

Sl. No.	Biochemical Tests	Isolates					
		1	2	3	4	5	6
1	Glucose	+	+	+	+	+	+
2	Sucrose	+	+	+	+	+	+
3	Galactose	+	+	+	+	+	+
4	Mannitol	+	-	+	+	+	-
5	Salicin	-	-	-	-	-	-
6	Dulcitol	-	-	-	-	-	-
7	Maltose	-	-	-	-	-	-
8	Lactose	+	+	-	+	-	+
9	Mannose	+	+	+	+	+	+
10	Sorbitol	+	-	+	+	-	+
11	Xylose	+	+	+	+	+	+
12	Dextrin	-	-	-	-	-	-
13	Rhamnose	-	-	-	-	-	-
14	Inulin	-	-	-	-	-	-
15	Arabinose	+	-	+	-	+	+
16	Cellobiose	+	+	+	+	+	+
17	Fructose	+	+	+	+	+	+
18	Trehalose	+	+	+	+	+	+
19	Malonate	-	-	-	-	-	-
20	Raffinose	-	-	+	+	+	-

Not a single isolate could be recovered from 33 mastitic milk samples which signified that the major pathogen causing mastitis is not *Pasteurella multocida* but other organisms like Streptococcus, Staphylococcus and Bacillus (Sarangi et al., 2009) which differs from the attributed fact that *Pasteurella multocida* causes mastitis in cattle in about 2-3 per cent of cases (Langoni et al., 2001 and Park et al., 1980)**.**

Detection of *P. multocida* by species and type specific PCR was one of the major objectives of the present study as it reduces time of diagnosis. Using the primer pair KMT1SP6-KMT1T7, approximately 460 base pair amplified product was obtained from all the *P. multocida* isolates (Fig. 1). All the isolates showed an amplified product of 760bp suggesting that all the isolates belonged to capsular type B. This is in contrast to previous report of Kar et al. (2003) who obtained capsular type A from Haemorrhagic Septicaemia cases from Odisha. Since amplified product was observed in colony PCR, by combining primers of PM-PCR with capsular typing primers both confirmation of presence of *P. multocida* and its capsular type can be known in a single reaction. Similar type of results has been reported by Dutta et al. (2001) and Townsend et al. (2000)**.** The isolates were also screened by HSB-PCR using primer sets KTSP61-KTT72 showing a distinct amplified product of 620bp (Fig. 2) emphasizing its use in rapid and specific diagnosis of haemorrhagic septicaemia. The results of PCR amplification are shown in Table 5.

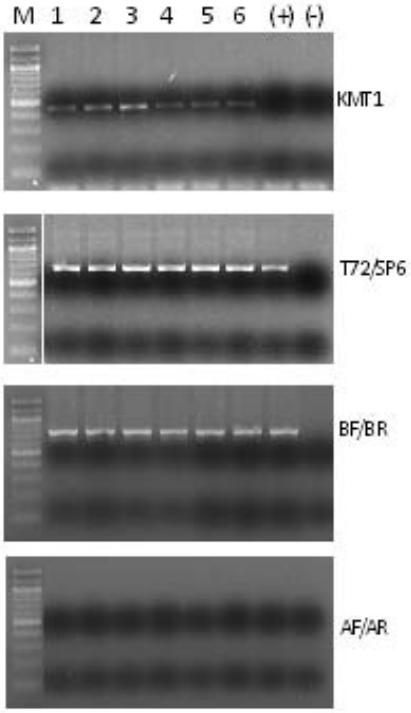

Fig. 1: Showing result of PCR amplification

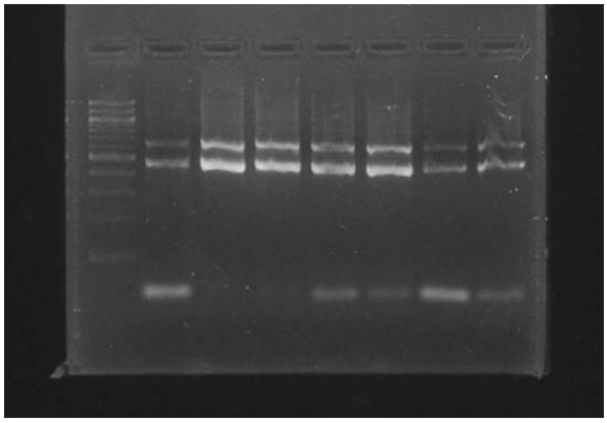

Fig. 2: Results of multiplex PCR

Table 5: Results of PCR amplification

Sl No	Isolate	PM-PCR	Serogroup A PCR	Serogroup B PCR	HSB-PCR	Multiplex PCR
1	1	+	-	+	+	++
2	2	+	-	+	+	++
3	3	+	-	+	+	++
4	4	+	-	+	+	++
5	5	+	-	+	+	++
6	6	+	-	+	+	++
7	P52	+	-	+	+	++

All the six *P. multocida* isolates were examined for sensitivity to 34 different antibiotics and the result suggests that isolates were highly sensitive to second and third generation cephalosporins like ceftriaxone, cefaclor, cephotaxime and cephalexin while cephadroxil, cefexime and ceftazidime were found to be 60 to 70 per cent effective. Among quinolone compounds moxifloxacin and enrofloxacin were found to be 80 per cent effective, whereas sparfloxacin, ciprofloxacin, ofloxacin, and pefloxacin revealed 60 to 70 per cent sensitivity. Gentamicin, tobramycin, amikacin, tetracycline, clotrimazole, carbenicillin, ampicillin, amoxicillin etc. showed 50 to 60 percent sensitivity while ticarcillin-clavulinic acid and chloromphenicol showed 80 to 90 per cent sensitivity. It is further observed that all the isolates were found to be 100 per cent sensitive to Piperacillin-tazobactum. But lowest sensitivity was noticed in case of oxytetracycline i.e. 34 per cent which corroborates with the study undertaken by Post et al. (1991) because. Amoxycillin and ampicillin have showed 50 per cent and 66 per cent sensitivity respectively and increased resistance to these antibiotics is due to production of resistance factor beta lactamase. Aminoglycosides particularly streptomycin revealed zero sensitivity, where as gentamicin and tobramycin revealed a sensitivity pattern of 50 per cent each. This is in contrast to Shayegh et al. (2009) who observed 96 per cent sensitivity against streptomycin and gentamicin. Low sensitive pattern to gentamicin observed in the present study could be due to non maintenance of proper blood concentration, improper schedule and dose leading to development of resistance. Amikacin, the next generation of amynoglycoside revealed a sensitivity of 50 per cent, which might be due to resistance conferred through indiscriminant use of gentamicin. About 80 per cent sensitivity of chloramphenicol was observed suggesting *P. multocida* isolates to be highly sensitive to chloramphenicol (Shayegh et al., 2009; Kumar et al., 2009). Quinolone compounds showed 66 to 83 per cent sensitivity. Among quinolone compounds enrofloxacin and ciprofloxacin are currently being used for veterinary practice with ciprofloxacin in particular is being used extensively for treatment of mastitis and HS cases. Moxifloxacin showed a higher sensitivity pattern as compared to others. These results are in accordance with some recent reports on antibiotic sensitivity pattern of bovine isolates of *P. multocida* (Onat et al., 2010; Sharma et al., 2010) Among cephalosporins, First generation of cephalosporins like cephalexin and cephadroxil revealed a variable pattern of sensitivity i.e. 100 per cent and 67 per cent respectively. This result disagrees with Parija (2003) who observed that cephalexin and cephadroxil have a sensitivity pattern of 10 per cent and 60 per cent only which might be due to strain variability. The second generation cephalosporin antibiotics like cephaclor and cefuroxime showed a sensitivity pattern of 100 per cent and 67 per cent and basing on the sensitivity pattern, it holds a better prospective for treatment of Pasteurellosis infection in near future. Third generation cephalosporins like ceftriaxone, cephotaxime and ceftazidime revealed a sensitivity pattern of 100, 100 and 83 per cent respectively and hence, these may prove as a better choice of antibiotic for treatment of HS cases as reported (Shram et al., 2010).

CONCLUSION

A total of six *P. multocida* isolates were recovered from 178 samples from suspected cases of haemorrhagic septicaemia from bovines. Preliminary biochemical and cultural characters were similar in all the isolates but sugar utilizing ability differed among the isolates. *P. multocida* species specific primer based PCR was found to be helpful for identifying all the isolates based on yield of an amplified product of approximately 460 bp in all the isolates and hence this PM-PCR assay appears to be the best tool in quick and specific diagnosis of pasteurellosis. PCR using capsular type specific primers as well as HSB specific primers revealed all isolates to be capsular type B suggesting multiplex PCR can be used to identify haemorrhagic septicaemia suspected cases in a single PCR reaction. In antibiogram study cephalosporins, quinolone compounds and chloramphenicol antibiotics were found to be better for treatment of haemorrhagic septicaemia cases.

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e-planet **14** (2): 78-85 (December 2016)

Phenotypic characterization of indigenous Hansli chicken of Odisha

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Date of receipt - 10. 10. 2016 Date of acceptance- 31. 12. 2016

ABSTRACT

A study was undertaken to investigate the phenotypic characteristics and morphometry of Hansli chicken of Odisha, wherein 350 adult chicken(160 males and 190 females) were taken for evaluation of qualitative traits and morphometry (i.e. shank length, shank width, shank circumference, keel length, breast angle, body girth, head width, body length, back length, neck length, height, thigh length, beak length) at 8th week, 12th week, 16th week and 20th weeks of age. The predominant plumage colour was black/red (49.3%) followed by black/golden yellow (31.25%) in male but the predominant plumage colour in female was black (80%). The plumage pattern was solid in both male and female birds. The predominant feather colour of neck/hackles was golden yellow (45.6%) in males and black colour (73.15%) in females. Sickle feather colours were mostly black in both males (78.12%) and females (78.4%). The predominant saddle feather colours were mostly golden yellow in males (43.12%) and black colour (76.8%) in females. All the morphological measurements were highly significant in male compared to female at 16th week, 20th week as well as in adult birds but only few morphological measurements were significant at 8th week and 12th week. The average shank length and circumference were 15.15±0.12 and 6.77±0.05 cm, and 12.07±0.07 and 5.33±0.02 cm in adult male and female birds respectively. The average keel length and thigh length were 15.13±0.11 and 24.26±0.02 cm and 12.59±0.06 and 19.49±0.21 cm in adult male and female birds respectively. The average body length and body weight were 57.98±0.43 cm and 3723.13±69.72 g and 47.62±0.17 cm and 2512.94±35.8 g in adult male and female Hansli birds respectively. From this study, it was concluded that the Hansli birds carried distinct qualitative and quantitative traits and would be further useful for conservation of this valuable genetic resource in future.

Key words: Characterization, Hansli chicken, morphometry, phenotype, traits

INTRODUCTION

Poultry sector plays a significant role in improving the socio-economic status of rural people by generating employment opportunity and augmenting family income particularly among landless labourer, small and marginal farmers and women in rural areas (Biradar et al., 2011). Local chickens play an important role for small farmers and contribute significantly to food security of households in rural and semi-urban communities (Abdelqader et al., 2007). The indigenous chickens were very hardy by which they are highly tolerate the harsh environmental condition and poor

husbandry practices without much loss in production (Dessie et al., 2011). Though indigenous poultry birds are less productive but have many valuable cultural and economic significance than the "exotic" chicken (Mangesha and Tsega, 2011). Propagation of exotic birds in rural areas for higher production leads to extinction of many native germplasms (Singh, 2009). Hence, the need of conservation and improvement of poultry genetic resources has been accepted worldwide. Characterizing local chicken types and their production systems is prerequisite for designing and implementing development and conservation programs (Qamashoui et al., 2014). Genetic characterization based on molecular assessment is a costly method and require high technology to evaluate genetic diversity (Hillel et al., 2003). Hence, many researchers are now using characterization method based on phenotypic traits, which is cost effective and also provides valuable information (Duguma, 2006). The indigenous poultry birds which are available in Odisha are Hansli, Gujuri, Dumosil, Vejaguda, Dhinki, Kalahandi, Phulbani etc. Among them Hansli chicken is predominantly reared in Mayurbhanj and its nearby districts in Odisha (Mohapatra et al., 2006). The people of Mayurbhanj district in Odisha have been traditionally rearing these birds for generation. They rear these birds for meat, egg as well as for fighting purpose. Hansli resembles Aseel to a great extent, but phylogenetic tree analysis indicated that Hansli is very much different from Aseel (Sahu et al., 2015). The present study was undertaken to phenotypically characterize indigenous Hansli chicken of Odisha, India by taking qualitative and quantitative morphological traits.

MATERIALS AND METHODS

The study was conducted at PG Department of Poultry Science, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar and Udala, Suliapada, Shyamakhunta, Kaptipada and Khunta block of Mayurbhanj district. A total of 30 Hansli bird owners were interviewed randomly from the selected blocks. A total of 350 birds (male 160, and female 190) were taken for morphological measurements (i.e. shank length, shank width, shank circumference, keel length, breast angle, body girth, head width, body length, back length, neck length, height, thigh length, beak length) at 8^{th} week, 12^{th} week, 16^{th} week, 20^{th} weeks of age and also adult birds. Qualitative traits i.e. plumage colour, head shape, shank feathering, skin colour were recorded following the recommended descriptor for chicken genetic resources (FAO, 2011). Comb type, shank length, shank colour, ear lobe colour, eye colours were recorded according to Cuesta (2008). Body length, back length, neck length, keel length, body girth, shank circumference and height of the birds were measured using a graduated tape to the nearest of 1cm accuracy. The shank length, shank width, thigh length, beak length, head width of the individual bird were recorded with the help of electronic digital callipers in cm. The breast angle of individual bird was recorded with the help of a breast meter to the nearest of one degree accuracy. For measuring the breast angle, the apparatus was placed posterior to the anterior edge of keel bone. Body weight was measured in kilogram using an electronic hanging scale (accuracy 0.01 g). All the data were analyzed by using (SPSS version 16).

RESULTS AND DISCUSSION

Qualitative traits

The features of qualitative traits and their distributions are presented in Table-1. The predominant plumage colour in male was black/red (49.3%) followed by black/golden yellow (31.25%) but the predominant plumage colour in female was black (80%). The plumage pattern was solid in both male and female birds. Yellowish featherless shank was observed in both male and female Hansli birds. The wattle colour was red and size of the wattle was medium (48.10%) and rudimentary (51.78%) in male but absent in female birds. The pea type comb (100%) was predominant in male and female. Ear lobe colour was red. Ear lobe was medium (67.85%) and small (32.14%) in male birds but small (100%) in female. Skin colour was dark pink in male 21.87%), female (20.6%) and light pink in male (78.13%), female (79.4%) birds. Eye

colour was pearl in both male and female which did not agree with the earlier report of Sarker et al. (2012); where they found the colour of eye was yellow in Aseel birds. In this study, egg shell colour was found mostly cream (50.27%) followed by light brown (45.8%) which did not agree with the earlier report of (Sarker et al., 2012), where they found egg colour was mostly light brown (77.78) in Aseel birds. Spur was well developed in male birds but was absent in female birds. Four toes present in both male and female birds. Plumage pattern was solid both in male female birds. The present study revealed that all the birds were having pea type comb but Sarker et al. (2012) reported few cases of strawberry comb and cushion comb (Everett, 2010) in Aseel birds.

Table 1. Qualitative traits and its frequency (%) in Hansli chicken

Traits	Characteristics feature	Male	Female
		Frequency in %	Frequency in %
Plumage colour	Black/Red	49.3	Absent
	Black/Golden yellow	31.25	Absent
	Black	15.6	80
	Grey	Absent	4.7
	Brown/Golden/red	Absent	11. 57
	White	Absent	2.89
	Light red	3.8	Absent
	Multi colour	Absent	1
Plumage pattern	Solid	100	100
Shank colour	Yellowish	100	100
Shank feathering	No feathering	100	100
Ear lobe colour	Red	100	100
Ear lobe shape	Medium	67.5	Absent
	Small	32.5	100
Eye colour	Pearl	100	100
Beak colour	Light yellow	100	100
Skin colour	Dark pink	21.87	20.6
	Light pink	78.13	79.4
Comb type	Pea	100	100
Comb colour	Red	100	100
Wattles size	Medium	48.75	Absent
	Rudimentary	51.25	Absent
Wattles colour	Red	100	Absent
Head	Flat	100	100
Spur (large)	Present	100	Absent
Toes	Four toes	100	100
Egg colour	Deep brown		2.79
	Light brown		45.8
	Cream		50.27
	White		1.10

Table 2. Distribution and frequency (%) of feather colour in Hansli chicken

Colour of Feather	Male						Female						
	Black	White	Red	Black and White	Black and Red	Golden Yellow	Black	White	Red	Brown	Black and White	Black and Red	Grey
Hackle feather	3.75	1.87	41.8	0	6.87	45.60	73.15	1.57	3.6	12.1	4.21	2.63	2.63
Sickle feather	78.12	3.125	9.35	3.75	5.62	0	78.40	0.52	6.3	10.52	1.57	1.57	1.05
Saddle feather	3.12	0	41.25	3.12	6.25	43.12	76.80	1.57	7.3	11.57	1.57	2.10	2.10
Breast feather	69.3	0	17.5	3.75	9.37	0	77.36	1.05	1.5	11.05	1.57	5.78	1.57
Wing Bow feather	79.37	0	8.75	0	7.5	4.37	75.78	1.05	3.1	10.0	2.10	2.10	5.78
Wing Bar feather	13.75	0	38.12	0	6.25	41.8	75.20	1.5	2.1	11.05	2.10	2.10	5.78
Wing Bay feather	76.25	0	10.62	0	7.5	5.625	76.80	1.5	2.1	10.0	2.10	2.63	4.73
Primary feather	43.12	3.75	38.12	3.75	6.87	4.37	45.20	1.5	4.2	28.9	2.63	8.42	8.94

Table 3. Morphological measurement (Mean±SE) at 8th week of age

Traits	Male	Female	SEM
Shank Length (cm)	7.31±0.06	7.20±0.06	0.065*
Shank width (mm)	8.54±0.13	8.47±0.12	0.063
Shank circumference (cm)	3.57±0.11	3.40±0.01	0.018*
Keel length (cm)	6.38±0.063	6.37±0.06	0.113
Breast angle (deg)	41.97±0.32	42.64±0.29	0.121*
Body girth (cm)	17.95±0.13	17.95±0.14	0.133
Head width (cm)	2.36±0.014	2.36±0.024	0.118
Body length (cm)	27.81±0.39	27.42±0.28	0.112
Back length(cm)	11.94±0.09	11.93±0.10	0.067
Neck length(cm)	9.78±0.09	9.74±0.089	0.063
Height (cm)	25.76±0.26	25.41±0.20	0.148
Thigh length (cm)	11.99±0.11	11.81±0.11	0.137*
Beak length (cm)	2.52±0.20	2.48±0.02	0.299

*indicates significant difference in a row ($p< 0.05$)

Table 4. Morphological measurement (Mean±SE) at 12th week of age

Traits	Male	Female	SEM
Shank length (cm)	10.33±0.12	10.32±0.08	0.083
Shank width (mm)	11.52±0.12	11.31±0.13	0.138
Shank circumference (cm)	4.37±0.02	4.31±0.03	0.030*
Keel length (cm)	8.71±0.07	8.67±0.07	0.030
Breast angle (deg)	50.94±0.29	50.13±0.36	0.134*
Body girth (cm)	24.67±0.16	24.55±0.15	0.128
Head width (cm)	2.83±0.01	2.80±0.01	0.134*
Body length (cm)	37.56±0.25	36.76±0.25	0.122*
Back length (cm)	16.42±0.12	16.07±0.14	0.073*
Neck length (cm)	14.22±0.11	14.11±0.10	0.082
Height (cm)	35.63±0.37	35.55±0.34	0.159
Thigh length (cm)	17.04±0.11	16.69±0.13	0.172*
Beak length (cm)	3.04±0.02	3.04±0.01	0.361

*indicates significant difference in a row ($p < 0.05$)

Table 5. Morphological measurements(Mean±SE) at 16th week of age

Traits	Male	Female	SEM
Shank length (cm)	12.57±0.13	11.32±0.07	0.078*
Shank width (mm)	13.41±0.11	12.48±0.13	0.138*
Shank circumference (cm)	4.83±0.04	4.52±0.03	0.031*
Keel length (cm)	10.36±0.09	9.67±0.07	0.043*
Breast angle (deg)	51.31±0.6	50.27±0.55	0.143*
Body girth (cm)	28.6±0.27	26.6±0.20	0.115*
Head width (cm)	3.14±0.02	2.97±0.01	0.151*
Body length (cm)	44.78±0.36	41.92±0.19	0.209*
Back length (cm)	19.86±0.18	18.36±0.15	0.074*
Neck length(cm)	16.71±0.16	16.08±0.09	0.099*
Height (cm)	42.82±0.66	39.48±0.4	0.215*
Thigh length (cm)	20.17±0.20	18.38±0.14	0.277*
Beak length (cm)	3.35±0.03	3.24±0.02	0.575*

*indicates significant difference in a row ($p < 0.05$)

Table 6. Morphological measurements (Mean±SE) at 20th week of age

Traits	Male	Female	SEM
Shank length (cm)	14.1±0.11	11.97±0.11	0.112*
Shank width (mm)	14.82±0.13	13.06±0.09	0.113*
Shank circumference (cm)	5.27±0.03	4.87±0.03	0.038*
Keel length (cm)	11.59±0.09	10.84±0.08	0.032*
Breast angle (deg)	55.19±0.43	53.39±0.36	0.093*
Body girth (cm)	31.03±0.38	29.04±0.25	0.132*
Head width (cm)	3.26±0.02	3.09±0.02	0.186*
Body length (cm)	48.75±0.36	45.06±0.61	0.163*
Back length (cm)	21.74±0.18	20.54±0.16	0.089*
Neck length (cm)	18.35±0.09	17.73±0.1	0.094*
Height (cm)	48.12±0.40	43.65±0.34	0.250*
Thigh length(cm)	21.58±0.16	19.77±0.18	0.381*
Beak length(cm)	3.59±0.02	3.44±0.02	0.365*

*Indicates significant difference in a row ($p < 0.05$)

Table 7. Morphological measurements (Mean±SE) of adult birds

Traits	Male	Female	SEM
Shank length (cm)	15.15±0.12	12.07±0.07	0.088*
Shank width (mm)	20.48±0.25	14.95±0.13	0.123*
Shank circumference (cm)	6.77±0.05	5.33±0.02	0.026*
Keel length (cm)	15.13±0.11	12.59±0.06	0.051*
Breast angle (cm)	66.19±0.36	63.27±0.38	0.159*
Body girth (cm)	40.00±0.39	32.92±0.16	0.255*
Head width (cm)	3.77±0.03	3.13±0.01	0.247*
Body length (cm)	57.98±0.43	47.62±0.17	0.225*
Back length (cm)	25.71±0.26	20.64±0.26	0.076*
Neck length (cm)	22.04±0.19	19.16±0.08	0.116*
Height (cm)	61.54±0.58	51.6±0.64	0.190*
Thigh length (cm)	24.26±0.22	19.49±0.21	0.393*
Beak length (cm)	3.76±0.02	3.38±0.02	0.448*
Body weight (g)	3723.1±69.72	2512.9±35.8	0.363*

*Indicates significant difference in a row ($p < 0.05$)

Feather Colour Distribution

Feather colour distribution and its frequency in Hansli male and female were presented in Table 2. The predominant feather colour of neck/hackles was observed golden yellow (45.6%) in males and black colour (73.15%) in female. Sickle feather colours were mostly black in both males (78.12%) and females (78.4%). The predominant saddle feather colours were mostly golden yellow in male (43.12%) and black colour (76.8%) in female. Breast feather colours were black (69.3%) in male and female (77.36%) birds. Wing bow feather colours were mostly black in both males (79.37%) and female (75.78%). The major wing bar feather colours were golden yellow (41.8%) in male and black colour in female (75.2%). The dominant primary feather colours were black in both sexes. The findings on hackle feather colours and saddle feather colours did not corroborate with the study undertaken by Sarker et al. (2012) on Aseel birds but findings of breast feather, wing feather and sickle feather colours were well matched with the study report of Sarker et al. (2012) in Aseel birds.

Morphometry

Different morphological measurements are presented in Table 3, 4, 5, 6 and 7 at 8^{th} week, 12^{th} week, 16^{th} week, 20^{th} week of age and in adult birds, respectively. At 8^{th} of age, shank length, shank circumference, thigh length and keel length were significantly higher in male compared to female birds but breast angle was significantly higher in female compared to male birds and the remaining traits such as shank width, keel length body girth, head width, body length, back length, neck length, height and beak length did not significantly differ in male and female birds. At 12^{th} week of age, shank circumference, breast angle, head width, body length, back length and thigh length were significantly higher in male compared to female birds and the other body parameters such as shank length, shank width, keel length, body girth, neck length, height, beak length were not significantly different between male and female birds. At 16^{th} week, and 20^{th} week of age as well in adult birds all the morphological measurements were highly significant in male birds compared to female birds. In this study, the male birds were having significantly longer shank length compared to female birds at all ages except 12^{th} week of age.

The mean value of shank length (cm) at 20^{th} week of age was (14.1 ± 0.11) in male and (11.97 ± 0.11) in female birds which was higher than the previous results of (Haunshi et al., 2011) in Aseel males (12.53 ± 0.90) and females (10.19 ± 0.60) at 40 week of age. The mean value of shank length (cm) in adult Hansli males (15.15 ± 0.12) and females (12.07 ± 0.07) were higher than the earlier research of Sarker et al. (2012) in Aseel male (12.79 ± 0.13) and female (10.21 ± 0.25) birds. Relatively lower value of shank length (9.52 ± 5.14) and keel length (8.40 ± 1.04) were reported by Chatterjee et al. (2007) in Aseel birds as compared to the present finding. The mean value of keel length (cm) of adult male (15.13 ± 0.11) and female (12.59 ± 0.06) birds were higher than the previous finding of (Sarker et al., 2012) in Aseel male (14.39 ± 0.19) and female (10.79 ± 0.23) birds. The average adult live weight in male and female birds were measured 3723.00 ± 69.72 g and 2512.94 ± 36.80 g, respectively.

The present finding on mature live weight agreed with the report of Sarker et al. (2012), where they found the adult body weight of Aseel chicken ranged between 2.0 to 3.7 kg. Relatively wider shank circumference was observed in male birds compared to female birds at all ages, which corroborated with the finding of Sarker et al. (2012).

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e-planet **14** (2): 86-98 (December 2016)

Butterfly diversity in Fakir Mohan University campus, Balasore, Odisha, India

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Date of receipt: 07. 09. 2016 Date of acceptance: 31. 12. 2016

ABSTRACT

Butterflies are one of the most predictable bioindicator organisms. During the one year of survey in Fakir Mohan University campus, a total number of 53 species of butterflies were identified belonging to five different families. Maximum numbers of species were from the family Nymphalidae followed by Pieridae. The abundance of butterfly species was observed to be maximum when the temperature was near the range of 28 ± 2^0 C. The variation in species richness was correlated with the climatic conditions as well as to the host plant interaction. The present study aims at the conservation of the butterflies and development of appropriate management strategies.

Keywords: Butterflies, predictable, bio-indicator, nymphalidae, pieridae, conservation

INTRODUCTION

The earth exhibits great diversity in the composition of its flora and fauna in different parts of the globe. India is considered a mega-biodiversity country where richness of plant as well as animal life is observed. Order Lepidoptera is regarded as one of the important components of biodiversity (New and Collin, 1991). The butterflies constitute the second largest group under the order Lepidoptera and the class Insecta having colorful wing patterns. Further, butterflies are good biological indicators of habitat quality as well as general environment health (Kocher and Williams, 2000; Larsen, 1988; Swachik et al, 2005), as many species are strictly seasonal and prefer only particular sets of habitats (Kunte, 2000). Thus, minor changes in their habitats may lead to either migration or local extinction (Blair, 1999; Kunte, 1997; Mennechez et al, 2003). The butterflies are known due to beautiful morphology and different colors of wings, and also have aesthetic value (Ali and Path, 2000). In nature many organisms are present that are solely responsible for the minute changes in surrounding biotic conditions and from their mode of adaption a biologist can simply access the variation in ecosystems. By this variation, a population may be damaged either partially or totally. Butterflies are good indicators for environmental assessment due to their sensitiveness, and they are directly affected by the alterations in their habitats. They are a well studied taxonomic group of insects. India is described as a “butterfly paradise” (Venkataraman, 2010). The country harbors 1504 numbers of butterfly species (Tiple, 2011); out of this some are endemic as well as globally threatened species. Studying of butterfly community needs various biotic as well as abiotic factors, which directly influence

their distribution patterns, i.e. humidity, temperature, wind, host plants, etc. The present study reveals the seasonal pattern and abundance of butterfly diversity in the study area.

MATERIALS AND METHODS

The Fakir Mohan University, situated in the Balasore district of Odisha State and established in 1999 in the fond memory of Fakir Mohan Senapati, renowned novelist, has a coverage area of 263 acres. The university is situated on 21° 32' 39.0552" North Latitude and 86° 48' 57.8772" East Longitude near the foothill of Niigiri mountain range. This area is diversified by various flora and fauna. The study site is mostly dominated by Mango tree (*Mangifera indica*), Custard apple *(Annona reticulata*), False Ashoka (*Polyalthia longifolia*), Guava (*Psidium guajava*), Indian jujube (*Ziziphus mauritiana*), Indian banyan (*Ficus benghalensis*), Peepal tree (*Ficusreligiosa*), Tamerin (*Tamarindus indica*), Arjun (*Terminalia arjuna*), Mexican oleander (*Thevetia peruviana*), Peacock flower (*Caesalpinia pulcherrima*), China rose (*Hibiscus* sp.) Banana (*Musa* sp.), Prickly poppy (*Argemone mexicana), Ixora Coccinea, Mimosa pudica*, etc, which are widely distributed and provide better host plants for both caterpillars and butterflies.

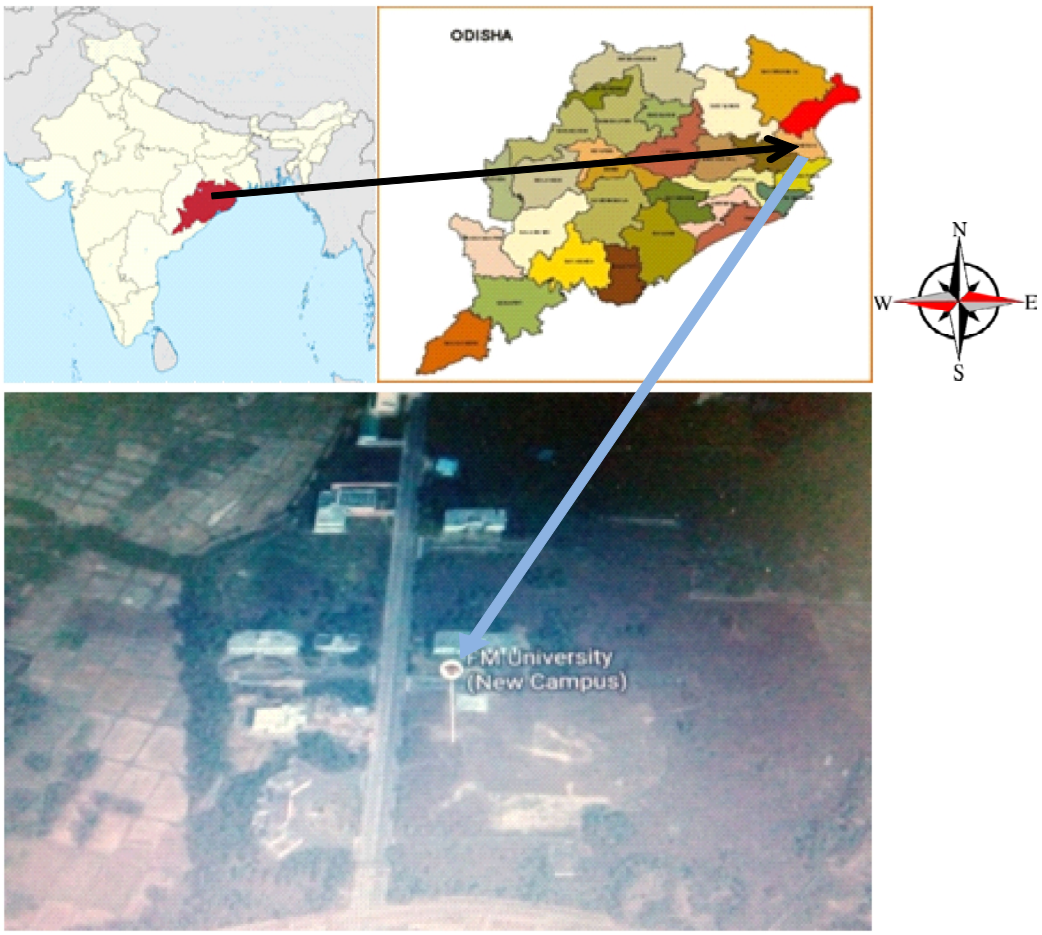

Fig. 1. Fakir Mohan University Map (Source: Google Map)

The survey was carried out with main focus on documentation of butterfly distribution around the campus. This was done at different sites of the campus by point count and line transects method from July 2015 to August 2016. The records were taken from sacred grove region, various departments periphery; Site 01: Department of Bioscience and Biotechnology, Population Studies, Sociology, Environmental Science; Site 02: Library garden; Site 03: Administrative garden; Site 04: Healthcare garden; Site 05: Guest house region; Site 06: Boys' hostel; Site 07: Ladies' hostel and Site 08: Staff quarters lines.

The data were collected during 09 hr to 11 hr and from 14 hr to 16 hr on alternate days. The maximum and minimum temperatures were noted. Photographs were taken by Nikon Coolpix S7000 camera and later compared with butterfly of India website and also by taking the help of field guides such as 'Butterflies of peninsular India' and 'The Book of Indian Butterflies' (Kehimkar,2008; Kunte,2000). Data sheets were prepared and noted during the whole survey period.

RESULTS

A total of 53 species of butterflies belonging to 5 families were recorded during the course of the study. The most common species were: Common mime (*Papilio clytia*), Common Lime (*Papilio demoleus*), Tawny coster (*Acraea violae*), Peacock pansy (*Junonia almana*), Common Indian crow (*Euploea core*), Common emigrant (*Catopsilia pomona*), Mottled emigrant (*Catopsilia pyranthe*), Plain tiger (*Danaus chrysippus*), Striped tiger (*Danaus* genutia), Commander (*Moduza procris*), Indian skipper (*Spialia galba*), Grass demon (*Udaspes folus*) and Rice swift (*Borbo cinnara*).

Out of the total number of species observed in the study area, the Family Papilionidae had 7 species (13%), Family Pieridae had 13 species (25%), Family Nymphalidae had 20 species (38%), Family Lycaenidae had 8 species (15%) and Family Hesperiidae had 5 numbers of species (9%).

The IUCN and WPA status of each family were recorded. From the survey, no IUCN threatened status species was identified. Some of the species not coming under the IUCN status are coming under the Wildlife (Protection) Act, 1972 under different schedules.

The most common family which occurred all the time during the survey was Nymphalidae. The maximum number of butterflies were recorded when the temperature was about 28±2°C. The flocks of butterflies were noticed during the morning and afternoon sessions. The maximum number of butterflies were noticed at the Sacred groove region and near the Guest house region, where the host plants were more in number.

Table 1. List of Butterflies recorded from F.M. University campus with their status

Common Name	Zoological Name	WPA Status	IUCN Status	Abundance
Family: Papilionidae				
Common Mime	*Papilio clytia*	I	-	C
Common Lime	*Papilio demoleus*	-	-	C
Common Mormon	*Papilio polytes*	-	-	C
Tailed Jay	*Graphium agamemnon*	-	-	R
Common Jay	*Graphium doson*	-	-	VR
Crimson rose	*Pachliopta hector*	I	-	C
Common Rose	*Atrophneura aristolochiae*	-	-	C

Family: Pieridae				
Common emigrant	*Catopsilia pomona*	-	-	VC
Mottled emigrant	*Catopsilia pyranthe*	-	-	VC
Common albatross	*Appias albino*	-	-	VC
Striped albatross	*Appias libythea*	I	-	VR
Yellow orange tip	*Ixias pyrene*	-	-	R
Common bush brown	*Mycalesis perseus*	-	-	R
Common grass yellow	*Eurema hecabe*	-	-	VC
Small grass yellow	*Eurema brigitta*	-	-	VC
Spotless grass yellow	*Eurema laeta*	-	-	C
Pioneer	*Belenois mesentina*	-	-	R
Psyche	*Leptosia nina*	-	-	R
Common jezebel	*Delias eucharis*	-	-	C
Common gull	*Cepora nerissa*	II	-	VR
Family: Nymphalidae				
Tawny coster	*Acraea violae*	-	-	VC
Common Coster	*Ariadne merione*	-	-	C
Common sergent	*Athyma perius*	-	-	R
Indian fritillary	*Argynnis hyperbius*	-	-	R
Common crow	*Euploea core*	IV	-	VC
Plain tiger	*Danaus chrysippus*	-	-	VC
Striped tiger	*Danaus genutia*	-	-	VC
Great eggfly	*Hypolimnas bolina*	-	-	C
Danaid eggfly	*Hypolimnas misippus*	I	-	VR
Common leopard	*Phalanta phalantha*	-	-	VC
Common three ring	*Ypthima asterope*	-	-	VC
Common four ring	*Ypthima huebneri*	-	-	C
Common sailer	*Neptis hylas*	-	-	R
Common castor	*Ariadne merione*	-	-	R
Angled castor	*Ariadne ariadne*	-	-	R
Lemon pansy	*Junonia lemonias*	-	-	C
Chocolate pansy	*Junonia iphita*	-	-	C
Peacock pansy	*Junonia almana*	-	-	C
Yellow pansy	*Junonia hierta*	-	-	VR
Grey pansy	*Junonia atlites*	-	-	VR
Family: Lycaenidae				
Common pierrot	*Castalius rosimon*	I	-	C
Striped pierrot	*Tarucus nara*	-	-	VC
Zebra blue	*Leptotes plinius*	-	-	C
Pale grass blue	*Psuedozizeeria maha*	-	-	C

Lesser grass blue	*Zizina otis*	-	-	VC
Yam fly	*Loxura atymnus*	-	-	VR
Pea blue	*Lampides boeticus*	-	-	-
Lime blue	*Chilades laius*	-	-	VC
Family: Hesperiidae				
Grass demon	*Udaspes folus*	-	-	C
Rice swift	*Borbo cinnara*	-	-	C
Common grass dart	*Taractrocera ceramas*	IV	-	C
Indian skipper	*Spialia galba*	-	-	C
Common red eye	*Matapa aria*	-	-	R

Abbreviations: WPA = Wild Life (Protection) Act, 1972; C = Common, VC = Very common, R = Rare, VR = Very Rare

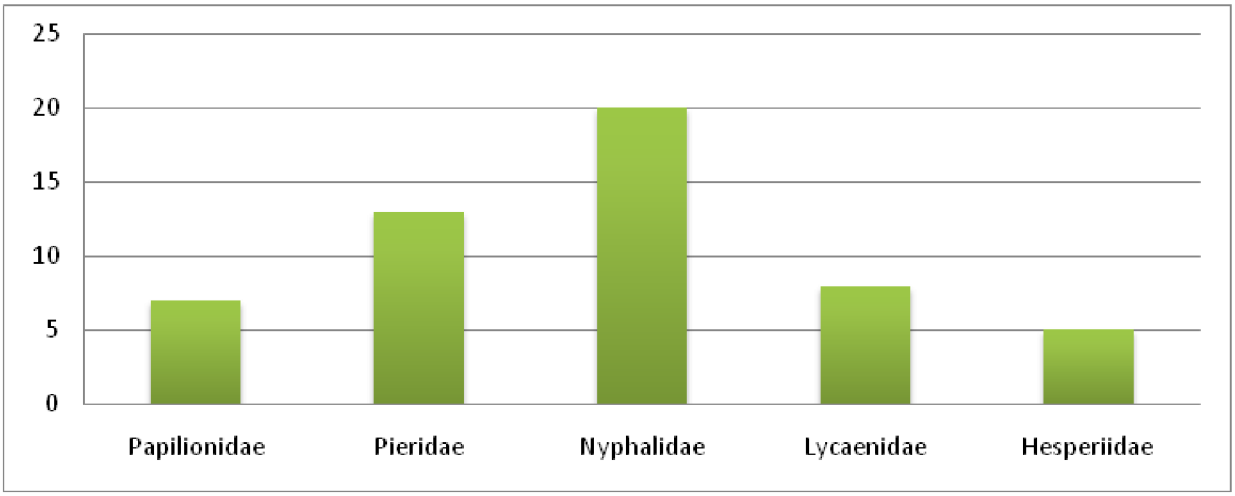

Fig. 2. Number of butterfly species in different families

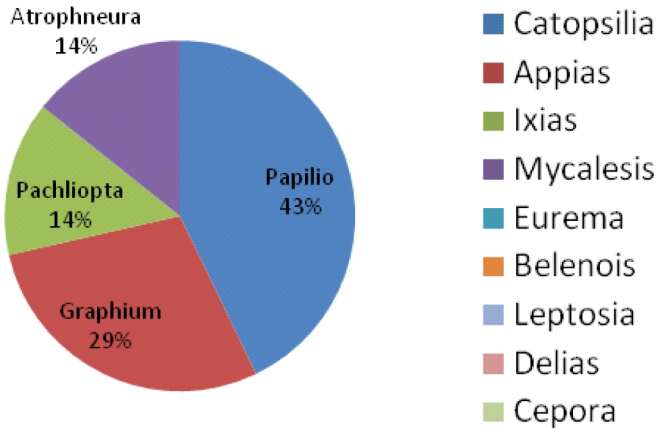

Fig. 3. Genus assimilation of Papilionidae family

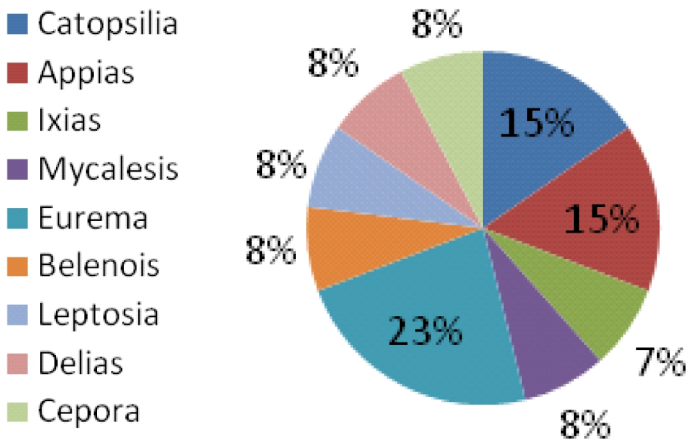

Fig. 4. Genus assimilation of Pieridae family

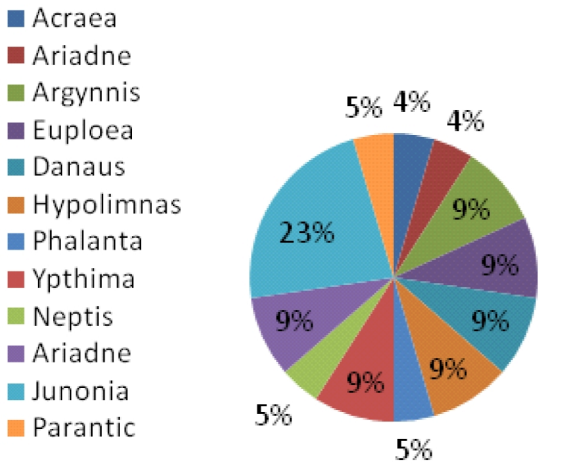

Fig. 5. Genus assimilation of Nymphalidae family

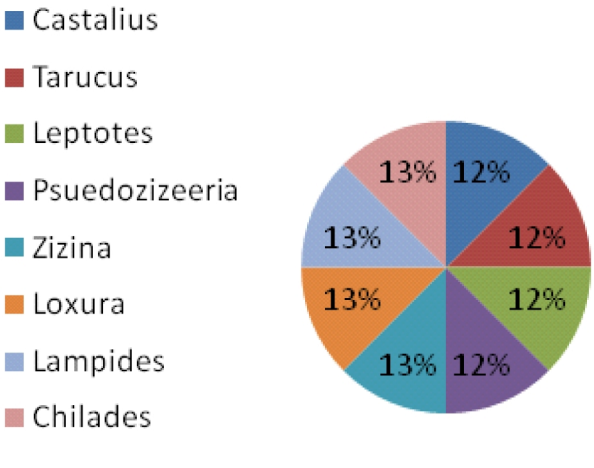

Fig 6. Genus assimilation of Lycaenidae family

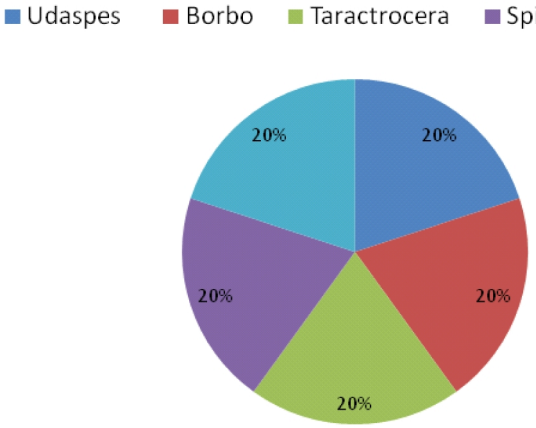

Fig. 7. Genus assimilation of Hesperiidae family

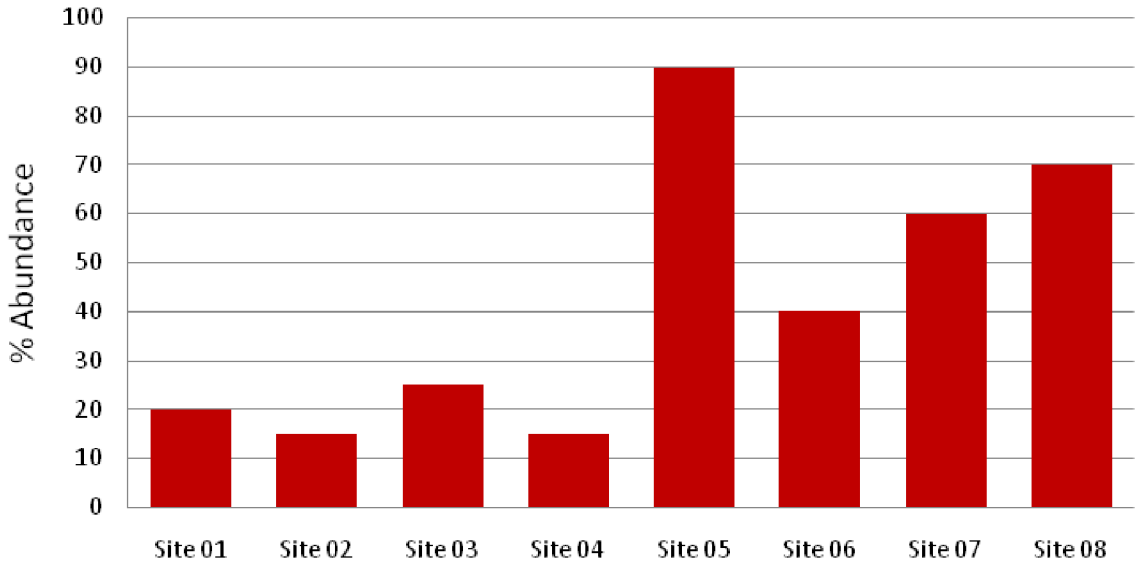

Fig. 8. Butterfly species showing richness at different sites

Fig. 9. Common moormon

Fig. 10. Common rose

Fig. 11. Lime butterfly

Fig. 12. Common gull

Fig. 13. Common emigrant

Fig. 14. Mottled emigrant

Fig. 15. Common grass yellow

Fig. 16. Spottless, grass yellow

Fig. 17. Common crow

Fig. 18. Common leopard

Fig. 19. Common tiger

Fig. 20. Striped tiger

Fig. 21. Tawny coster

Fig. 22. Grey pansy

Fig. 23. Chocolate pansy

Fig. 24. Peacock pansy

Fig. 25. Great eggfly

Fig. 26. Common Castor

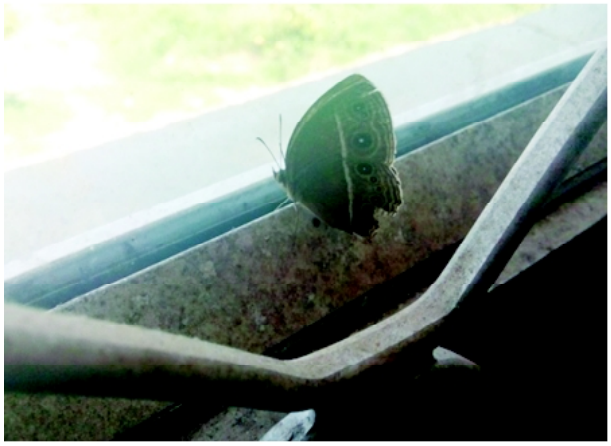

Fig. 27. Common Bush brown

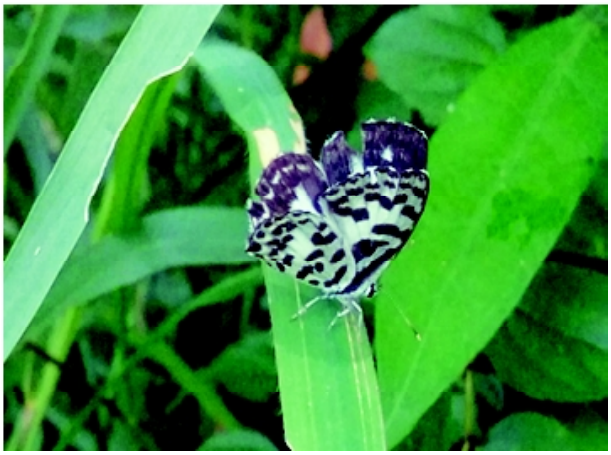

Fig. 28. Common pierrot

Fig. 29. Gram blue

Fig. 30. Rounded pierrot

Fig. 31. Psyche

Fig. 32. Lime blue

Fig. 33. Yam fly

Fig. 34. Grass demon

Fig. 35. Indian skipper

Fig. 36. Common red eye

RESULTS AND DISCUSSION

The current study on the butterfly diversity indicates that the F M University campus is a good habitat for their abundance. The butterflies are playing vital roles in the assaying of the environmental quality for a specific biotope (Kunte, 2000). Butterflies are solely responsible for pollination in different floral species (Mahendra et al., 2013; Sharma and Joshi, 2009). The diversity of butterflies varies in different parts of India (Larson, 1988). This one year study has demonstrated that the patterns of butterfly distribution inside the campus area show seasonal variation. The peak observation of butterfly density was recorded during the month of July. The peak distribution of butterflies was observed during the month of July due to abundance of host plants for the monsoon rain. Various abiotic factors are responsible for the diversity of butterflies distribution pattern and communal distribution is depending upon the seasonal impact over the density (Hammer et al., 2005). These abiotic factors influence a lot on their diversity (Amala et al., 2011; Arya et al., 2014; Barlow et al., 2007; Ghosh and Siddique, 2005; Kunte, 2000, Swachik et al., 2005). Temperature is the most important factor for the insect life (Kumar, 2013). The variation in butterflies must be due to the abundance of host plant, because both butterflies and caterpillars are dependant over the respective host plants. They are host plant specific, thus the diversity of butterfly is purely related to the host plant association (Gowda et al., 2011; Solman, 2004). Out of them, the butterflies are seasonal and site

specific (Padhy et al., 2006). The surrounding grassland, patches of forest land and heavy vehicle prohibition make a silence zone which directly affect over the climatic condition of the campus (Das and Parida, 2015). The landscape also influences over the diversity pattern; pressure of urbanization and presence of agricultural fields lead to habitat degradation of butterfly species (Clark et al., 2007). Various researchers recorded butterfly species from various locations (Arya et al., 2014; Bhuyan et al., 2002; Bora and Meitei, 2014; Nair et al., 2014; Saha et al., 2015; Sayeswara, 2014).

The highest number of species belongs to Nymphalidae family, which topped the list due to the polyphagous nature for which this family survived in all biotic condition (Das and Parida, 2015). The butterflies are basically migrating from the adjoining village and from Nilgiri foothills for searching of food sources (Raut and Pendharkar, 2010). The Pieridae family is having attractiveness towards the flowers (Mali et al., 2014).

The well managed biotic condition, avoiding of habitat degradation and development of various floral species will lead to butterfly conservation by giving full effort.

CONCLUSION

The current study has shown that a good diversity of butterfly exists inside the university campus and this diversity is due to plenty of availability of host plants, which create a opportunistic ground for the development of butterflies. This rich faunal and floral diversity is well interacted. The environment of the campus is not much polluted and this preliminary study needs to be followed by a detailed study in future for the butterfly faunal assessment.

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