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Prospects of plant based edible vaccines in combating COVID-19 and other viral pandemics: A review

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ABSTRACT

Many pathogenic viruses have always posed serious threats and challenges to human existence by causing epidemic and pandemic outbreaks and losses of millions of lives every year. Recently, a novel corona virus, COVID-19 has been creating havoc with huge numbers of casualties the world over. Vaccination with a suitable vaccine is the hope for the survival of humans on earth. Vaccines are used as prophylactic as well as curative measures against viral diseases and are effective in eradicating deadly pathogens. Conventional vaccines though effective but have high production costs, involve tedious purification processes and have bio safety issues, requiring time-consuming tests and procedures for commercial production whereas plant-based vaccines offer several advantages over the conventional systems such as ease of production, suitable folding of foreign protein, target protein stability, higher yields, storage, and safety. Plant based edible vaccines can play a major role in immunization to control the viral diseases across the world, especially in poor and developing countries as these products can be easily scaled up for millions of doses within a limited period and can save millions of life who now die for lack of access to the traditional vaccine. The edible vaccine has the potential to solve the problem of bioterrorism by immunizing against a wide range of different dreaded viruses and can save the earth from any future epidemic and pandemic. It could become the best and cheapest alternative to conventional vaccines, for which there is a great need to strengthen research and development activities in this promising area so that in near future children may get immunized by foods instead of painful immunization through needles.

Key words: Bioreactors, COVID-19, human viral diseases, pandemic, plant edible vaccines

INTRODUCTION

The last ten decades has witnessed many deadly viral pandemics. The first and most severe pandemic known as Spanish influenza, occurred in 1918 and was caused by an H_1N_1 influenza A virus (IAV) strain (Jordan, 2019). Approximately 500 million people were infected, and 50 million people died during this pandemic. The second pandemic, known as "Asian influenza," occurred in 1957, was caused by an H_2N_2 IAV strain, and resulted in nearly 1.1 million deaths worldwide (Glezen, 1996). The third pandemic, known as "Hong Kong flu," occurred in 1968 and was caused by an H_3N_2 IAV strain, resulting in nearly one million deaths worldwide (Viboud, 2005). The last 40 years has been no exception, as the world witnessed the emergence and reemergence of viral

outbreaks, of which Human Immunodeficiency Virus (HIV) in 1981, Severe Acute Respiratory Syndrome Corona virus (SARS-CoV) in 2002, H_1N_1 influenza virus in 2009, Middle East Respiratory Syndrome Corona virus (MERS-CoV) in 2012, Ebola virus in 2013 and the Severe Acute Respiratory Syndrome Corona virus-2 (SARS-CoV-2) in 2019, are noteworthy (Zappa et al., 2009; Bloom and Cadarette, 2019; Grubaugh et al.,2019; Cascella et al., 2020; Roychoudhury et al.,2020). The emergence of deadly viruses and their global outbreaks pose threats to the public health and economy of the world. The COVID-19 pandemic is proving to be an unprecedented disaster, especially in terms of the health, social and economic aspects. Countries with both high and low-income groups are facing catastrophic consequences (Bong et al., 2020). Viral diseases like smallpox, polio, tetanus, measles, etc. are restricted by vaccination (Altindis et al., 2014). Vaccination is the process by which the body is made ready to face and fight off new infections by improving immunity for a long duration through antibody production so that future infections can be warded off (Malik et al., 2011). Thus, higher priority is given to vaccination for prevention and control of dreaded viral diseases. Traditional vaccines consist of inactivated or attenuated pathogens and are not entirely safe (Chan and Daniell, 2015). Live attenuated and killed viruses carry the risk of reverting to virulence, as well as other drawbacks in terms of antigenic variability between species, low levels of immunogenicity, and possible gene transfer to wild-type strains (Adeniji and Faleye, 2015; Burns et al., 2014). An outbreak of type 2 vaccine-derived polio in Nigeria was first detected in 2006, became endemic in Africa, and persists today (Famulare and Hu, 2015). This large poliomyelitis outbreak, caused by type 2 circulating vaccine-derived poliovirus (cVDPV2), began in 2005 in northern Nigeria (Burns et al., 2013). Additionally, they are inefficient in producing a protective response at mucosal surfaces such as the lungs and intestinal tract, the actual sites where disease agents enter the body. Therefore, new approaches are needed to improve current vaccines. The use of transgenic plants to produce subunit vaccine proteins has been developed as an alternative platform for the large-scale production and delivery of vaccines to induce protective immune responses via the mucosal immune system (Daniell et al., 2009). Plants offer several major advantages in vaccine generation, including low-cost production by eliminating expensive fermentation and purification systems, sterile delivery, and cold storage/transportation. Most importantly, oral vaccination using plant made antigens confers both mucosal (IgA) and systemic (IgG) immunity (Streatfield, 2006; Rybicki, 2010; Salyaev et al., 2010; Obembe et al., 2011). The rapid spread and frequent pandemic of severe contagious viral diseases such as HIV, SARS, Ebola, COVID-19, etc require extremely rapid development and comprehensive distribution of edible vaccines against potentially deadly, novel pathogenic viruses. The review describes the importance of plant-derived vaccines, various advantages, research and developments, challenges and future prospects in human immunity against viral diseases.

PRESENT GLOBAL CRISIS ON COVID PANDEMIC

About COVID-19

The 21st century witnessed a severe disease outbreak due to spread of a novel form of corona virus i.e. SARS CoV-2 that causes COVID-19. Corona viruses (subfamily Coronavirinae, order Nidovirales) are common human pathogens. They are enveloped, positive-sense, single-stranded RNA viruses that belong to the family Coronavirdiae, and are known to cause acute respiratory, hepatic and neurological diseases with varying severity in humans as well as animals (Denis et al., 2020 and Zumla et al., 2016). SARS-CoV-2 is transmitted via inhalation or direct contact with droplets from infected people. It has an incubation period ranging from 2 to ≥14 days.(Denis et al., 2020 and Zumla et al., 2016). On 11 March 2020, WHO characterized SARS-CoV-2 as a pandemic situation. In China, the total number of confirmed cases reported is 84,520 including 4645 deaths. The USA has reported the highest number of cases (1,525,186) and deaths (91,527) (mortality rate >12%). The spread of COVID-19 in India, which is a neighbouring

country to China, where the number of active cases (118,447) is increasing on a daily basis but with a low death rate i.e. ~3.0% (Sharma et al., 2020). It was first reported during December 2019 from Wuhan, China. Department of Health, Republic of China confirmed it on 23.01.2020 (WHO, 2019). Slowly, it spread to different other countries. Majority of population of Wuhan Province were affected and there were large number of casualties. China took strong steps and restricted flights from and to Wuhan and imposed lockdown and shutdown. Health examination guaranteed, affected people were quarantined, people were advised to use mask and maintain social distance. If no mask, harsh punishment was implemented. Stadiums were turned into quarantine and health service centres. General public accepted and cooperated with the new set of regulations and ensured quick immunization of its citizens. With the above set up measures, they were able to control the disease significantly. From late December, 2019 to early 2020, many people flew from China to Italy, Germany, Spain, America, Brazil, India and other countries becoming carriers and spreading the disease in rest of the world. As of now, around 195 countries of the world have been affected with this dreaded disease. Globally, as on 07 June 2021, near about 173 millions confirmed cases of COVID-19 including more than 3.72 million deaths have been reported (WHO, 2021). Later on, due to the severe impact of COVID-19 and subsequent reduction of body immunity with steroid led treatment, a fatal black fungus (Mucormycosis) disease reappeared and affected the people of some countries causing huge casualties. Its white and yellow variants have also been reported in various parts. Mucormycosis happens to be a fungal infection that causes blackening or discoloration over the nose, blurred or double vision, chest pain, difficulties in breathing and oozing of blood during coughing etc. With exposure to mucor mould, commonly found in soil and air, this black fungus occurs (Govt. of Odisha, 2021). Amphotericin B liposome injections are recommended against black fungus infection (ICMR, 2021). The COVID-19 infection rate and death cases of different regions of the world reflects the transmissibility and virulence nature of this virus. On 22 May 2020, there were 4,995,996 confirmed cases of SARS-CoV-2 including 327,821 deaths in 216 countries, and the number is increasing worldwide. The graphical presentation from WHO data is herewith presented to analyze the increase in global deaths (Fig.1). It's impact was such that as on June 2021 there were 179513309 confirmed cases, 3895661 confirm deaths. The vaccine doses administered were 2624733776 (WHO, 2021).

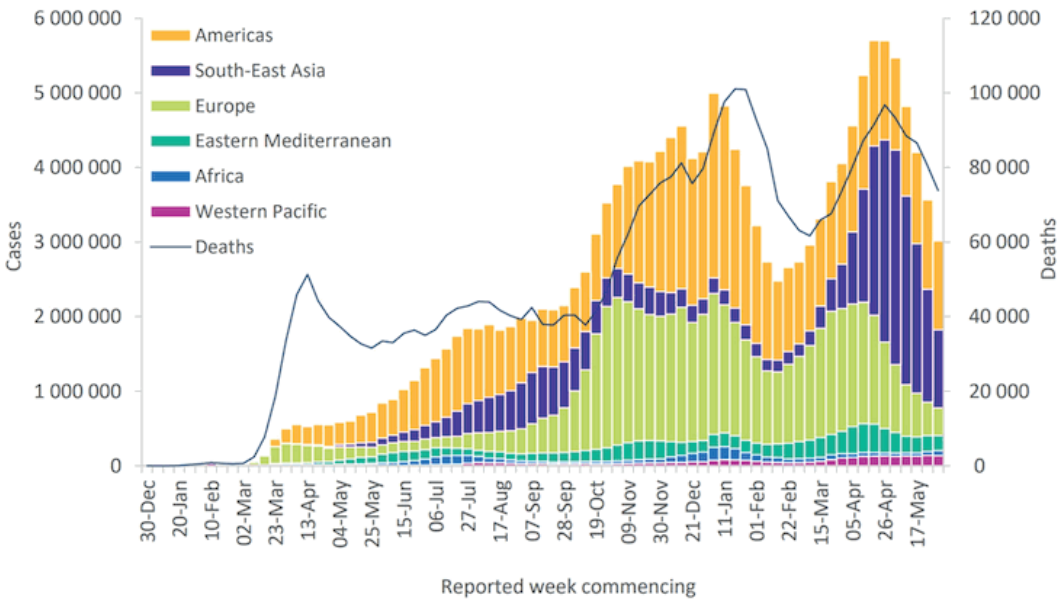

Fig. 1. COVID-19 cases and deaths in different world regions

The WHO, in collaboration with national authorities, institutions and researchers, routinely assesses if variants of SARS-CoV-2 result in changes in transmissibility, clinical presentation and severity, or if they result in changes in the implementation of public health and social measures (PHSM) by national health authorities. Systems have been established to detect "signals" of potential Variants of Concern (VOCs) or Variants of Interest (VOIs) and assess these based on the risk posed to global public health. Table 1 lists currently designated global VOIs and VOCs.

The second wave in India during April-May, 2021 was an emerging lineage of SARS-CoV-2 variants B.1.617, particularly its sub-lineage B.1.617.2 (a.k.a. delta variant). B.1.617 variant which was first reported in India in October 2020, contained mutations in the key spike protein regions involved in interactions with the host and induction of neutralizing antibodies and the strain evolved to three more sub-lineages B.1.617.1-3. Recent studies of the Delta variant in the United Kingdom of Great Britain and Northern Ireland suggest a possible increased risk of severe disease, and support previous observations of increased transmissibility. An analysis comparing Delta and Alpha variant confirmed cases in the United Kingdom from 29 March to 20 May 2021 showed the Delta variant was associated with a possible increased risk of hospitalization (hazard ratio 2.61, 95%CI 1.56-4.36), and an increased risk of emergency care attendance or hospitalization (hazard ratio 1.67, 1.25-2.23) within 14 days of specimen collection, as compared to the Alpha variant (WHO, 2021).

More than thirty million children throughout the world do not receive even the most basic immunizations each year. As a result, a large proportion of these children die from viral diseases that are fully vaccine-preventable. To save these children, the idea of large scale production of edible vaccines for various diseases can provide the opportunity of best solution.Virus evolution is expected, and the more SARS-CoV-2 circulates, the more opportunities it has to evolve (WHO, 2021).

Table 1 : SARS-CoV-2 Variants of Concern (VOCs) and Variants of Interest (VOIs)

WHO label	Pango lineage	GISAID clade	Next strain clade	Earliest documented samples	Date of designation
Variants of Concern (VOCs)					
Alpha	B.1.1.7	GRY (formerly GR/501Y.V1)	20I/501Y.V1	United Kingdom, Sep-2020	18-Dec-2020
Beta	B.1.351	GH/501Y.V2	20H/501Y.V2	South Africa, May-2020	18-Dec-2020
Gamma	P.1	GR/501Y.V3	20J/501Y.V3	Brazil, Nov-2020	11-Jan-2021
Delta	B.1.617.2	G/452R.V3	21A/S:478K	India, Oct-2020	VOI: 4-Apr-2021 VOC: 11-May-2021
Variants of Interest (VOIs)					
Epsilon	B.1.427/ B.1.429	GH/452R.V1	20C/S.452R	United States of America, Mar-2020	5-Mar-2021
Zeta	P.2	GR	20B/S.484K	Brazil, Apr-2020	17-Mar-2021
Eta	B.1.525	G/484K.V3	20A/S484K	Multiple countries, Dec-2020	17-Mar-2021
Theta	P.3	GR	20B/S:265C	Philippines, Jan-2021	24-Mar-2021
Iota	B.1.526	GH	20C/S:484K	United States of America, Nov-2020	24-Mar-2021
Kappa	B.1.617.1	G/452R.V3	21A/S:154K	India, Oct-2020	4-Apr-2021

Monoclonal antibodies, a recent advancement

In India, compared to 2020, the daily infections being reported this year are arising in alarming rate; over 50 lakhs patients across India in first fortnight of May 2021 indicating the gravity of 2nd wave of infections. Unlike the characteristics of the infections, reported in 2020, this year doctors and experts have pointed out that the younger population and more members in one family are being infected. Amidst the sudden rise of cases, uncertainties over the behavior of the virus and with only least research, experts advise people to get themselves vaccinated immediately and urging the government to expand the vaccine coverage across all age groups (Ranjan et al., 2021). Pharmaceutical company like CIPLA has recently produced the mono-clonal antibodies, a synthetic protein against treatment of present COVID variant. It is basically cocktailing of 12 mg Casirivimab mixed with 12 mg of Imdevimab. It is a single shot drug given through intra-veinous transfusion. After the injection, the patients are advised for 1 hour hospital observation. It is reported that if this drug is given within 24 hours of onset of symptoms of COVID-19, then there is every possibility for the patient to recover. If the patients are already hospitalized, and the patients' lungs and vital organs are already affected significantly by COVID virus attack, then this injection does not have any role to play and not recommended to be administered (FDA, 2020).

2 DG, the anti-COVID drug

Recently DRDO, Government of India has developed a drug i.e. 2DG (2-deoxy-D-glucose), an analogue of glucose, which is available as ORS pouch and is easily consumed through water. This drug is recommended to the Corona affected patients (moderate to severe), mostly those who are hospitalized. All patients beyond 18 years age group can take this except patients with high blood pressure and high blood sugar. This drug is said to work wonders in such patients and they are recovering fast with reduced days of hospitalization (DRDO, 2021).

Prophylactic measures against COVID-19

In post pandemic corona devastation, many countries have started developing vaccine to protect their citizens. China state owned company Sinopharm jab develop by the Beijing Institute of Biological Product which got approval from WHO. The WHO had previously only approved vaccine made by Pfizer, Astra Zeneca, Johnson & Johnson and Moderna. Then another vaccine at China was developed by Sinovac. In the meanwhile, in other countries Pfizer Inc., an American multinational Pharma's vaccine against covid-19 was started in use. Then Russia produced Sputnik (Ranjan et al., 2021). In India, Serum Institute of India produced Covishield and later Bharat Bio-tech produced Covaxin which were of 2 doses recommendations. The guidelines for 2nd dose covishield injection has been notified by Health and FW Department (Govt. of Odisha, 2021).

Keeping in view of this, this particular review is undertaken to explore the possibilities of plant derived vaccines for easy and economic usage to protect and safeguard the human life against corona viral pandemic in the present context. The authors urge upon the scientific community of the world to give a serious thought to expand the research outlets to reach into some kind of conclusive recommendations in these areas.

ADVANTAGES OF PLANT BASED EDIBLE VACCINE

Conventional vaccines produced from attenuated pathogens involve the synthesis of antigenic proteins via mammalian cell culture which is easily prone to contamination with harmful pathogens. Vaccine production through microbial system invites the possibility of endotoxin contamination. When cell culture and transgenic animals are used to make vaccines, the contamination possibly arises with viruses, prions, and oncogenic DNA. Hence, biosecurity and biosafety issues need to be addressed to cultivate these pathogenic agents (Mishra et al., 2008). Vaccine construction in several cases has been hampered because of varying or mutating strains of the pathogen, antigen drift, antigenic shift, and other unrevealed mechanisms. Such regulations make it difficult to select the actual peptide sequence to prime the immune system since the peptide sequences of the individual strains will all

be different. Even if successfully produced, these commercial vaccines have a specific expiry period and need refrigeration facilities, thereby enhancing control, storage, transportation, and distribution costs. Vaccine degradation after acid digestion in the stomach is another concern (Khan et al., 2019). Most of the available vaccines are associated with several problems including safety issues due to vaccine-associated side effects in human subjects. Thus, the World Health Organization in 1990 presented the task of finding cheap methods of oral vaccine production that must be safer and do not need refrigeration requirements. Dr. Charles Arntzen of Arizona State University first put forward the idea of edible vaccines which pose an interesting alternative in overcoming some of the constraints of traditional vaccines. They offer cost-effective, easy to administer, storable, widely-acceptable, and bio-friendly vaccine delivery system, particularly in developing countries. Edible vaccines are generally antigen-expressing plants, where the edible part of a plant is genetically modified to express the antigens, thereby eliciting an immune response upon consumption, and serving as a factory for vaccine manufacturing (Doshi et. al., 2013). The various advantages of plant derived edible vaccine in comparison to the conventional vaccine are given in Table 2.

Table 2. Comparison between the conventional and plant derived edible vaccines

Conventional/Traditional vaccines	Plant based edible vaccines	References
Comprised of weakened, live attenuated or killed pathogen	Comprises of plasmid / vector carrier system or metal particles containing small segment of target DNA sequence.	Mercenier et al., 2001; Taylor and Fauquet, 2002.
Injected intramuscularly or Subcutaneously thus painful immunization procedures	Given orally i.e. needle-less vaccination thus attractive and easier for children	Streatfield ,2005; Mishra et al., 2008.
Inefficient in producing a protective response at mucosal surfaces	Efficient in producing a protective response at mucosal surface.	Yuki and Kiono, 2003; Streatfield, 2006.
Possess residual virulence	No residual virulence	Streatfield, 2006; Lal et al., 2007; Mishra et al., 2008.
Need extensive safety precaution	Have a wide of safety.	Daniell et al., 2001; Altindis et al.2014; Chen,2015; Concha et al. 2017.
Production difficulty and larger cost	Relative ease of production and cost effective	Giddings et al.2000; Streatfield, 2005; Nochi et al., 2007; Govea-Alonso et al., 2014.

MODE OF ACTION OF EDIBLE VACCINE

Edible vaccines contain DNA fragments from the original pathogen. These fragments code for a protein, that is usually a surface protein of the pathogen. This is responsible for eliciting the body's immune response. Most pathogens enter through the mucosal surface lining the digestive, respiratory, and urino-reproductive tracts which are collectively the largest immunologically active tissues in the body. The mucosal immune system (MIS) is the first-line defense as it is the area where human pathogens initiate their infection and the most effective site for vaccination against pathogens (Tacket and Mason, 1999). Hence, the success of an edible vaccine requires the induction of the mucosal immune system. Induction of a mucosal immune response starts with the recognition of an antigen by specialized cells called M-cells. These cells are localized in the mucosal membranes of lymphoid tissues such as Peyer's patches within the small intestines. The M-cells channel the antigen to underlying tissues where antigen-presenting cells

internalize and process the antigen. The resulting antigenic epitopes are presented on the APC surface, and with the assistance of helper T cells activate B cells. The activated B cells migrate to the mesenteric lymph nodes where they mature into plasma cells and migrate to mucosal membranes to secrete immunoglobulin-A (IgA). Upon passing through the mucosal epithelial layer towards the lumen, the IgA molecules complex with membrane-bound secretory components to form secretory IgA (sIgA). Transported into the lumen, the sIgA interacts with specific antigenic epitopes and neutralize the invading pathogen (Walmsley and Arntzen, 2000). Orally administered vaccines are particularly efficient at stimulating local mucosal immune responses at the intestinal surface, and the integrated nature of the mucosal immune system also allows other mucosal sites to be primed (Ruedl and Wolf, 1995)

RESEARCH AND DEVELOPMENTS IN EDIBLE VACCINES FOR HUMAN USE

The first plant-made vaccines (PMVs) were described by Curtiss and Cardineau in 1990 (Kirk et al., 2005). The first demonstration of expression of a vaccine antigen in plants was the *Streptococcus mutans* surface protein antigen A (SpaA) in tobacco in 1990. This research resulted in the first patent (US patent No. 5,654,184) related to the plant-based vaccine technology (Curtiss and Cardineau, 1997). Chimeric plant viruses were proven effective as carrier proteins for vaccinogens in 1994 after rabbits raised an immune response against purified chimeric CPMV particles expressing epitopes derived from human rhinovirus 14 (HRV-14) and HIV-1 (Porta et. al., 1994). Antigenicity of the resulting vaccinogen was demonstrated through mice feeding trials using the native gene for comparison. The synthetic gene increased antigen accumulation in leaves and tubers by 3-14-fold in comparison to wild-type transgene expression (Mason et al., 1998). Modelska et al. (1998) were the first to detect a mucosal immune response after oral induction with a plant virus-derived vaccinogen. It is well documented that delivery of a plant-derived vaccine to a mucosal tissue induces both a mucosal and a systemic immune response (Haq et al., 1995, Mason et al., 1996, Arakawa et al., 1998, Koo et al., 1999). In 1998, the first human trial of the edible vaccine was carried out with raw potato expressing a part of *Escherichia coli* toxin that causes diarrhoea. It was for the first time proved by the National Institute of Allergy and Infectious Diseases (NIAID) that significant immunogenicity can be induced safely by an edible vaccine. Brennan et al., (1999) further characterized the immune response induced by mucosal delivery of a plant-derived vaccine. During 2000, antigens of the Norwalk virus (that causes diarrhoea) were expressed in potato (Tacket et al., 2000). First-generation plant-based vaccines were produced against the influenza virus, human papillomavirus, and norovirus by modifying PVX or TMV. In second-generation plant-based vaccines, deconstructed viral vectors devoid of different viral elements needed for its replication and infectivity were used. Recombinant viral vectors with heterologous coat protein, with cell surface presentation of foreign antigen in the viral coat protein and sub-genomic promoters etc. were more stable, environmentally safe, and provided high yield.

In humans, plant-based edible vaccines have been found protective for human respiratory syncytial virus (F protein), hepatitis B (HBsAg), measles (H protein), Japanese encephalitis virus (envelope protein E), Norwalk viral gastroenteritis (virus capsid protein), anthrax [protective antigen domain IV, PA(dIV)], human papillomaviruses (HPV 16 E7 protein, HPV major capsid protein L1), tetanus for both human and animals (antigen TetC), rabies in farm, wild animals and humans (glycoprotein and nucleoprotein epitopes), human immunodeficiency virus (HIV), influenza etc. (Streatfield, 2006; Mishra et al., 2008; Daniell et al., 2009; Buonaguro et al., 2010; Lugade et al., 2010; Rybicki, 2010; Salyaev et al., 2010; Obembe et al., 2011; Rukavtsova et al., 2011; Shoji et al. 2015; Hayden et al., 2012).

The concept of using engineered or transgenic plants to produce and deliver subunit vaccines was introduced by Charles Arntzen (Mason et al., 1992). Potato and tobacco were used as model organisms initially in the development of

plant-based vaccines. Tobacco plants are a good model for evaluating recombinant proteins and can be harvested several times in a year and their leaves which are major sources of biomass. However, due to the composition of a high level of toxic alkaloids, it causes more toxicity (Tiwari et al., 2009; Doshi et al., 2013). The potato was used as a model plant in edible vaccine production since it was easy and efficient to transform, tuber-specific promoters could be used to express transgene/antigen gene, the out crossing risk was low, clonal propagation to produce stable transgenics was possible, tubers could be eaten (cooked), were used in the food industry, affordable and tubers could be stored for long periods without refrigeration. The major limitation is that, it needs cooking which can denature antigen and decrease immunogenicity (Arakawa et al., 1998; Hafiz and Eyob, 2015). Later, many different crops i.e. tomato, maize, rice, soybean, spinach, lupin, lettuce, etc are selected for research and development of an edible vaccine (Table 3).

Table 3. Developments of edible vaccines in different plants against human viral diseases

Sl. No.	Vaccine/ Antigen	Pathogen/ Viral diseases	Crop	Reference
1	Hepatitis B surface antigen	Hepatitis B	Tobacco	Mason et al.1992; Valdes et al. 2003; Huang et al.,2007, 2008
			Potato	Thanavala et al., 1995; Domansky et al., 1995; Richter et al., 2000; Youm et al. 2010
			Lettuce	Hayden et al.2012; Czyz et al. 2014
			Maize	Arakawa,1998;
			Tomato	Richter et al. 2000; Lou et al., 2007; Wang and Li, 2008
			Carrot	Zhao et al. 2002
			Lupin leaves	Pniewski et al., 2006,2011; Waghulkar, 2010
			Rice	Qian et al. 2008
			Banana	Guan et al.2010
			Cherry tomatillo	Gao et al. 2003
2.	Rabies virus glycoprotein and nucleo protein	Rabies	Tobacco	Hooper et al.1994; Arango2008; van Dolleweerd et. al.,2014
			Tomato	McGarvey et al., 1995
			Spinach	Modelska et al., 1998
			Potato	Yusibov, 2002
			Maize	Loza-Rubio et al,2008
3.	Norwalk virus capsid protein	Norwalk virus causing Gasteroenteritis	Tobacco	Mason et al. 1996
			Potato	Mason et al. 1996; Tacket et al, 2000; Yusibev et al. 2002
			Banana	Carter et al. 2002
			Tomato	Zhang et al., 2006,
4	Herps	Herps	Soybean	Zeitlin et al. 1998
5	Respiratory Syncytial viral G and F protein	Respiratory Syncytial virus	Tobacco	Belanger et al.2000
			Tomato	Sandhu et al. 2000

6	Paramyxovirus surface protein hemagglutinin	Measles	Tobacco	Huang et al 2001
			Tobacco, potato, rice and lettuce	Webster et al., 2005
7	Rotavirus VP6 protein	Rotavirus	Potato	Yu and Langridge, 2001; Yu-Zang et al., 2003; Wu et al., 2003, Li et al., 2006
			Tobacco	Yang et al., 2011; Pera et al., 2015
8	HIV antigen	AIDS	Tobacco	Marusic et al., 2001; Zhang et al. 2002, Meyers et al., 2008; Gonzalez-padabe et al. 2011; Rubio-infante et al. 2015
			Potato	Horn et al., 2003
			Spinach	Karasev., 2005
			Tomato	Shchelkunov et al., 2006
			Lettuce	Govea-Alorso et al., 2013
			Carrot	Lindh et al., 2014
9	Human papillomavirus E7 and L1 proteins	Cervical cancer	Potato	Franconi et al., 2002; Biemelt et al., 2003; Warzecha et al., 2003
			Tobacco	Liu et al., 2013
			Tomato	Monray-gracia et al., 2014
10.	Recombinant vaccinia virus B5	Smallpox	Spinach	Yusibev et al., 2002
			Tobacco and collard	Golovkin, 2007
11	SARC-CoV 2 Protein	SARS	Tomato and tobacco	Pogrebnyak et al., 2005
12.	Dengue virus glycoprotein	Dengue	Tobacco	Kim, 2009 and 2015
13.	Ebola immune complex	Ebola virus	Tobacco	Phoolcharoen, 2011; Fulton, 2015
14.	Influenza vaccine	Influenza	Tobacco	D'Aoust et al., 2010; Shoji et al., 2015; Ward et al., 2014; Pillet et al., 2015

Tomato plant grows quickly, cultivated broadly, heat-stable, and high vitamin A composition may boost immune response. Antigen-containing powders can be filled into capsules without having any requirement of special facilities for storage and transportation. However, it has demerit as spoils easily; hence cannot be stored for over a long period (Renuga et al., 2014). Bananas are sterile so the genes do not pass from one banana to another which is the main reason why bananas are a good choice for an edible vaccine. The tropical climate is suitable for growing bananas. It does not need cooking. Proteins are not destroyed even if cooked and they can be eaten as raw. It is inexpensive, can be grown widely in developing countries, grow quickly, and have a high vitamin A content which boosts immune response. The disadvantages

are that the trees take 2-3 years to mature. A transformed tree takes about 12 months to bear fruits, spoils rapidly after-ripening (William, 2002). Maize plants generate a protein that is used to develop the hepatitis B virus vaccine. It is cheaper and does not need to be refrigerated. A major disadvantage of this vaccine is to be cooked for the use which causes degradation of proteins (Arakawa et al. 1998). Rice plant has been reported that a strain of rice can serve as a vaccine and last for more than a year and a half at normal room temperature. It is used as pediatric food because of the low level of allergenic potential but grows slowly and requires specialized glass house conditions (Hafiz and Eyob, 2015).

Generally, vaccine development has six-phases according to the Center for Disease Control and Prevention (CDC), USA. These are exploratory, preclinical, clinical development, regulatory review and approval, and finally manufacturing and quality control (Baylor, 2016). In the exploratory phase, research and development on synthetic or natural antigens or weakened strains of the pathogenic virus are carried out to treat or prevent disease. In the pre-clinical phase, tissue culture or cell cultures and animal testing are undertaken to verify the effectiveness of the edible vaccine to provide immunity. In the third phase i.e. in clinical development, a proposal describing the research findings required to be submitted by the vaccine manufacturing firm to the sanctioning authority conducting clinical trials. After getting the approval to conduct clinical trials, human testing or trials are conducted in 3 stages i.e. in phase-I, the candidate vaccine is administered to a small group of people (<100) to know the safety. Phase-II involves a larger group in many hundreds to know about safety immunogenicity, immunization schedule, dosage, etc. A larger subject group of thousands are covered in Phase III trials where side-effects, safety, and effectiveness of the candidate vaccine is assessed. This is followed by regulatory review and approval where an application for a license for manufacturing by the firm is scrutinized for approval. The next step is manufacturing the vaccines and finally quality control to monitor the performance, safety, and effectiveness of the vaccine.

Plant-derived vaccines have been developed for many human diseases such as hepatitis B, Human Immunodeficiency Virus (HIV), rabies, etc. More than 25 vaccines are licensed for use in humans and many more in the development pipeline (Yusibov and Rabindran, 2008). In March, 2018, Medicago Inc. conducted phase III clinical trials to develop a flu vaccine in tobacco and is expected to be launched in the market by 2020-2021. In 2018, researchers from the University of Nottingham, Malaysia, launched a project to develop a plant-based vaccine against dengue fever, caused by Aedes mosquitoes. Researchers from Arizona State University's (ASU) Bio-design Institute developed a norovirus vaccine from the tobacco plant in 2018. Other than tobacco, many edible crop plants are also used such as the dengue virus vaccine produced in lettuce through chloroplast transformation in 2016. Medicago Inc., Prodigene, Rubicon lab, Agr Genet, Rhone-Merieux, iBio Inc., Icon Genetics-GmbH, Creative Biolabs, etc. are involved in plant-based vaccine development (Mishra et al., 2008). Some of the edible vaccine patents are also described in Table 4.

The Queensland University of Technology, Australia plans to use the genome sequence information of *Nicotiana benthamiana* to use the plant as a bio-factory to produce antibodies, vaccines, and therapeutics, to develop protein-based diagnostic products in bulk quantities at a low cost against COVID-19 or similar viruses or pathogens. The Canada-based biopharmaceutical company Medicago Inc. is experimenting on the transient expression of SARS-CoV-2 virus S protein, using a virus-like particle (VLP) grown in *Nicotiana benthamiana* to develop a potential vaccine against the coronavirus disease that has now reached a global pandemic level. Universities and institutes from several countries including the US, Germany, UK, South Africa, South Korea, Mexico, and Thailand are working in the molecular farming field, investing efforts and establishing partnerships and collaborations for production of vaccine/ treatment for COVID-19.

Table 4. Patents on edible vaccine technologies for human viral disease

Sl. No.	Patent holder	Claims
1.	University of Texas	Hepatitis B core antigen recombinant vaccine
2.	Prodigene	Production and transfer of recombinant antigen to plant cells through the plasmid-vector system; production of vaccine for hepatitis and transmissible gastroenteritis virus
3.	University of Philadelphia	Rabies vaccine expressed in tomato plant
4.	Biochem; Rhone-merieux	Transgenic plant used to produce rabies vaccine
5.	Biosource	Plant viral vector with potential as anti-HIV vaccine
6.	Rubicon Lab	Retrovirus expressed in animal or plant cells useful as virus and cancer vaccines
7.	Agr genet	Edible vaccine for human rahino virus

Transient transformation approaches are rapid (expression within a week) while the regeneration of a stably transformed plant takes up to 3 months, which is not suitable for addressing a fatal, exponentially growing pandemic such as COVID-19. Though, concept and methodology are concise, there is only a limited number of edible vaccines that are approved, tested and commercialized. (Parvathy, 2020).

FUTURE CHALLENGES

The major challenges of edible vaccine production are: (i) the selection of antigen and plant expression host, which will ensure the safety of the vaccine produced and its thermo-stability, (ii) consistency of dosage, and (iii) manufacturing of vaccines according to Good Manufacturing Practice (GMP) procedures (Laere et al., 2016). Therefore, the challenges facing plant-based-vaccine development include technical, regulatory, economic aspects, and public perception. Among technical challenges to be considered, the crop should provide ample biomass for the accumulation of a sufficient quantity of the antigenic protein.

The main regulatory concern is the expression of antigens in plants. Targeting transgene expression via a tissue-specific promoter may reduce regulatory concerns. Not all the vaccine candidate proteins are highly immunogenic in plant tissues. The secondary metabolites found in plants may compromise the ability of the candidate vaccine protein to induce immunity. Among regulatory challenges, issues relevant to any genetically modified crop that have to gain regulatory approval from the USDA, FDA, and/or EPA apply equally to vaccines generated in edible plant parts. Allergenic reactions to plant protein glycans and other plant antigens are challenging issues. It has been suggested that plant-derived recombinant proteins or antibodies may have increased immunogenicity or allergenicity, as compared to mammalian counterparts along with few side effects such as toxicity on the central nervous system, cytokine-induced sickness, and autoimmune diseases. Another problem is that the glycosylation pattern of plant and human protein is different which may affect or alter the functions of vaccines (Streatfield, 2005).

There are chances of contamination of recombinant proteins by pesticides, herbicides, mycotoxins, or potentially toxic factors because some plant species contain numerous toxic alkaloids and other secondary metabolites. All these factors invite regulatory constraints and uncertainties for approval as a human drug. The stability of vaccines in fruits is uncharacterized. Moreover, there may be weakening of the medicinal property and denaturation of vaccine proteins in the case of cooked foods. Evaluation of dosage requirement and proper maintenance of dosages are difficult, since the consistency of dosage may vary within the plants of the same species, from fruit to fruit and from generation to generation due to the size and ripeness of the fruits or plants

(Sharma and Sood, 2011). Fruits like tomato and banana do not appear in the market in fixed or standard sizes so that it is quite difficult to optimize the dosage. It is also quite difficult to evaluate the required dosage for every patient. To determine the right dosage, one needs to consider the person's weight, age; fruit/plant size, ripeness, and protein content. The amount to be taken is critical, especially in infants, who might spit it after taking, may eat a part of it, or may take it and throw it up later. The levels of innate and adaptive immune responses generated in different individuals may vary, based on the types of antigens being exposed in the body. Between two patients with different body weight as well as their age, the dosage of plant-derived vaccine required will be different. If this issue is not monitored carefully, an immunological tolerance will be induced when the patient is overdosed, while too low a dose would fail to induce antibodies. The development of immune-tolerance to the vaccine protein or peptide is therefore a major concern (Concha et al., 2017). Edible vaccines are dependent on plant stability as certain foods cannot be eaten raw (e.g. potato) and needs cooking that cause denaturation or weaken the protein present in it (Moss et al., 1999). Variable conditions for the edible vaccine are also a major problem. The potato-containing vaccine to be stored at 4°C could be stored for a longer time, while a tomato does not last long. Thus, these vaccines need to be properly stored to avoid infection through microbial spoilage (Merlin et al. 2017). Another challenge would be in dealing with diseases caused by multiple serotypes (dengue) or by rapidly mutating organisms (HIV, influenza). Though, the plant edible vaccines are a lucrative option in the field of vaccination, several potential issues need to be addressed via exhaustive research and development to use this area of health care for greater benefits (Raychoudary, 2020).

Some of the techniques to overcome these limitations are (i) optimization of the coding sequence of viral genes for expression as plant nuclear genes, (ii) expression in plastids, (Ruf et al., 2001) (iii) plant viruses expressing foreign genes (Nemchinov et al. 2000) (iv) coat-protein fusions, (Modelska et al.,1998) (v) viral-assisted expression in transgenic plants(Mor et al., 2003) and (vi) promoter elements of bean yellow dwarf virus with reporter genes GUS (β-glucuronidase) and GFP (green fluorescent protein), substituted later with target antigen genes. Antigen genes may be linked with regulatory elements which switch on the genes more readily, or do so only at selected times (after the plant is nearly fully grown), or only in its edible regions. Exposure to some outside activator molecules may also be tried. Although edible vaccines fall under genetically modified plants, it is presumed that these vaccines will avoid controversy, as they are intended to save lives.

Currently, it has not been studied that all the approved vaccines will work against the newly mutated variant of COVID-19 that are continuously evolving. The SARS-CoV 2 Spike protein can be used to develop a plant derived edible vaccine against the COVID-19 as previous work on SARS-CoV vaccines have shown that the Spike glycoprotein (S) is the main inducer of neutralizing antibodies in respiratory diseases. The Spike (S) protein gene or subunit of Spike like S1 subunit can be cloned into a plant expression vector and the desired plant like tomato, cucumber or lettuce can be transformed. The developed transgenic plants can be used as salad and easily delivered orally and immunized the human against the newly emerged virus and can develop herd immunity against the COVID-19 as well as other respiratory pathogenic viruses of the same order/family.

CONCLUSION

Plant-derived edible vaccines are safer with numerous advantages over the conventional vaccines such as easy to administer, readily acceptable vaccine delivery system, easy to store and are cost-effective. These can play a major role in immunization control of the viral diseases across the world, especially in poor and developing countries. These products can be easily scaled up for millions of users within a limited period. The development of a suitable edible vaccine is of great importance to prevent and control the spread of the virus. The edible vaccine has the potential to solve the problem of bioterrorism by immunizing against a wide range of different dreaded viruses and can save the earth from any future epidemic and pandemic. Currently, it appears that almost every country all over the World urgently feels the need of designing new vaccines against COVID-19 and other dreaded viruses as fast as possible to save human

life. Hence, there is a lot of scope for research and development in the field of the edible vaccine in the forthcoming era. The day is not far off when children may be immunized by foods instead of painful immunization through needles and agricultural food vaccines might save millions of life who now die for lack of access to the traditional vaccine. "Let food be thy medicine"-Hippocrates. The conclusive remark: Till now, although hundreds of years have been passed, research in entire world could not be able to eradicate the viral deaths and address the pandemic issues in right earnest and hence, the future children's health and safety are always at risk. Thus, urgently we should focus on developing edible vaccine to create a broad spectrum resistance continuously and naturally through food.

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Glyphosate resistance in rice: An overview

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ABSTRACT

Productivity of rice is influenced by a number of biotic and abiotic factors. Among these, weed accounts as a major constraint for rice production. It competes with the associated crops for water, soil nutrients, space, and light resulting in drastic reduction in crop yield. Manual and mechanical weeding incurs huge cost and often becomes impracticable, especially in areas with heavy weed infestation. However, non-selective herbicide particularly glyphosate proved to be a feasible solution to eradicate weed infestation, but it's off target movement to rice negatively influences plant growth, survival and seed yield. Therefore, development of herbicide resistance in rice turns to be the major focus in rice breeding. In the present study, the authors presented a detailed review of the weed management along with mode of action, weed resistance, genetic basis of glyphosate resistance, scope for exploring natural resistance, screening methodology and possible ways for development of glyphosate resistance in rice to confer selectivity and enhance crop safety and production.

Key words: Genetics, glyphosate resistance, mechanism, mode of action, natural resistance, rice breeding

INTRODUCTION

Rice (*Oryza. sativa* L., AA type genome, genome size 430 Mb) is an important annual cereal crop which provides foods nearly two-third of the world population. It is evident that rice production need to be increased to keep pace with the ever-increasing population. Among several biotic stresses, weed stands to be one of the major impediments in rice production (30-40% reduction in yield). Weed management by intercultural operations incur a huge cost towards cultivation (Prasad et al., 2019). Now-a-days the pre-emergence (alachlor, butachlor, pendimethalin and oxyfluorfen) and post emergence (propaquizafop, quizalofop ethyl, fenoxaprop-p-ethyl and imazethapyr) weedicides are often used to control the menace of weed infestation in rice fields. Outcrossing between rice and weedy grasses is generally low and such a chance event can be alleviated by application of herbicides at least at 4-5 leaf-bearing stage (14 DAT) to make the field free from weeds sufficiently ahead of flowering. Glyphosate has been used as the most effective herbicide to control weeds in rice cultivation areas in the world. It is a systemic broad-spectrum post emergence herbicide for weed control in glyphosate resistant crops (Senseman, 2007). Besides, it is widely used to indiscriminately kill annual and perennial weeds in heavily infested farmyards and such candidate herbicide is not potentially fit for application in rice fields as off target movements of glyphosate adversely affect growth of the rice resulting drastic reduction in yield. In this context, exploring natural herbicide resistance (NHR) to glyphosate in cultivated rice can be a novel approach to enhance selectivity and crop safety. Studies on natural resistance to weedicides in rice germplasm are limited (Nayak et al., 2020). Therefore, it is worthwhile to screen the available vast collection of rice germplasm resources for natural resistance to glyphosate.

USE OF HERBICIDES TOWARDS WEED MANAGEMENT

Weed is the major biotic stress in rice production leading to a yield loss in the range of 30- 40% (Abeysekara, 2011). They compete with crop plants in the field and negatively influence the crop yield quality and quantity along with survival of the plants. Weed control using herbicides is the most popular method among farmers and it allows economically viable weed control providing cost-effective method in the production of agricultural crops (Juraimi et al., 2013). In this context, herbicide-resistant rice has the potential to improve the efficiency of weed management. The common methods used to manage weeds include cultural, mechanical, biological, and chemical means. However, an integrated approach using a variety of methods in combination is reported to provide most successful weed management in rice fields (Tu et al., 2001).

MODE OF ACTION OF GLYPHOSATE ON WEEDS

In plants, glyphosate disrupts the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate(EPSP) synthase. The resulting deficiency in EPSP production leads to reductions in aromatic amino acids and other crucial plant metabolites (Duke and Powles, 2009) that are vital for protein synthesis and plant growth. Action of glyphosate begins immediately as soon as it coats the leaves, but it requires 4 to 20 days to completely kill the plants. The herbicide works the best when air temperature is above 15.5ºC and plants receive no rainfall or water for at least two days. It affects net CO_2 assimilation rate, transpiration rate, stomatal conductance, and internal CO_2 concentration in plants. Depending on the amount of damage sustained, plants may recover from glyphosate injury. Physiological injuries following glyphosate application in rice includes multiple shoots and roots from the internodes, leaf curling and discoloration, reduced exertion of panicles from flag leaf, and malformed inflorescence (non-emergence of panicle, aborted panicle, bleached lemma and palea).

WEED RESISTANCE TO GLYPHOSATE: A RISK FACTOR

The dreaded risk for commercialization of glyphosate resistant transgenic rice is the possibility of transfer of genes for herbicidal action to wild and weedy relatives leading to increased weed invasiveness and drastic reduction in yield (Kumar et al., 2008). Gene transfer between cultivated and wild or weedy rice is reported to form hybrids. The weedy rice hybrids could have more fitness ability for survival than cultivated rice even after glyphosate treatment. Besides, this may serve as source of gene flow owing its cross compartibility with related graminaceous weeds though at lower rate. The likelihood of such gene transfer is especially high in India owing to sympatrical distribution of the cultivated rice and its relatives and overlapping of their flowering period. Besides, indiscriminate use of glyphosate is likely to contribute evolutionary pressure for spontaneous induction of herbicide resistance in weed species. In fact, the resistant weeds are reported to be frequently evolved by modifying amino acid composition on the substrate binding site of EPSPS under high glyphosate selection pressure (Fartyal et al., 2018).

GENETIC BASIS AND MECHANISM FOR GLYPHOSATE RESISTANCE IN RICE

Herbicide resistance is the inherited ability of a plant to survive the herbicide application at a dose normally lethal to the normal population of the same species, while herbicide tolerance is the inherent ability of a species to survive and reproduce after herbicide treatment at normal concentration. Resistant weeds can often survive application of herbicide at rates that are much higher than the recommended dose. Possible mechanisms include over-expression of target enzymes, mutational change to altered target enzymes (mutant form) for reduced affinity to glyphosate, detoxification (metabolic degradation) of the herbicide and its reduced translocation and increased sequestration (partitioning to vacuole) to safe guard cells from harmful effects. Usually herbicides kill the weeds by either inhibiting or degrading the functional enzymes involved in metabolic pathway related to growth and photosynthesis. Glyphosate is

a structural analogue of phosphoenolpyruvate (PEP) and it blocks the Shikimic acid pathway by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the sixth enzyme in the shikimate biosynthesis pathway, resulting biosynthesis of essential aromatic amino acids (tryptophan, tyrosine and phenyl alanine) and subsequently phenolics, lignins, tannins and other phenyl-propanoids. Induced mutational variants for EPSPS gene leading to amino acid substitution at multiple active sites using site directed mutagenesis, and transgenics containing glyphosate resistant gene from bacterial source or other crop plants have been reported to offer glyphosate resistance in rice (Salas et al., 2012). Zhai et al. (2020) explored glyphosate stress-responsive microRNAs, lncRNAs, and mRNAs in rice using genome-wide high-throughput sequencing. Besides, spontaneous/ natural mutation of ESPS gene in local land races during the long span of domestication, and glyphosate resistant gene flow from weedy/red rice can not be ruled out. *Oryza spontanea*- the weedy rice, was in fact resulted from natural hybridization of *Oryza sativa* with either *Oryza nivara* or *Oryza rufipogon* (Tripathi et al., 2011).

SCOPE OF NATURAL TOLERANCE TO GLYPHOSATE IN RICE

Recently, more attention has recently been given to the naturally occurring glyphosate-sensitive EPSPS enzymes, as newer mutations providing increased glyphosate tolerance have been identified (Gherekhloo et al., 2017; Tian et al., 2015). Overexpression of improved mutant version of EPSPS gene has been reported to show increased field level glyphosate tolerance and higher grain yield in rice (Achary et al., 2020). Besides, 12 varieties (Bg359, At362, Bw364, Ld365, Bg366, Bg369, Bg379-2, Bg403, Bg454, Pachcha peruma, Kalu heenat and Kurulu thuda) had shown natural resistance to optimum concentration (0.5 g l^{-1}) of glyphosate (Ekanayaka et al., 2017) which might be due to spontaneous variation arose during the process of long period of domestication. Among these, At 362 and Ld 365 revealed survival percentage ≥ 70% while Bg 300 was shown to be most sensitive to Glyphosate with very low survival percentage (15%). Similarly, in an another report cv. Zambales was shown to be the most tolerant to glyphosate herbicide with highest survival rate and low severity index followed by Kalipao, Innaano and Kilay (among a set of 100 genotypes) which have moderate survival rate and low severity rate upon spaying of the crop with glyphosate based herbicide (locally called as 'Round-up') at 14 DAT (Palanog et al., 2015). This allows the use of such herbicide for weed control without effects on rice itself. Hence, there is scope for exploitation of natural variation for glyphosate tolerance in rice.

SCREENING TECHNIQUES TOWARDS GLYPHOSATE TOLERANCE IN RICE

A suitable glyphosate concentration can be determined using few rice varieties at varying glyphosate concentrations (0.25 g l^{-1}, 0.5 g l^{-1}, 1 g l^{-1}, 1.5 g l^{-1}, 2 g l^{-1}, 2.5 g l^{-1} and 3 g l^{-1}). LD_{50} based on survival percentage was worked out to be 0.5g l^{-1} and any variety with ≥50% survival percentage was arbitrary considered as resistant to glyphosate application (Ekanayaka et al., 2017). Subsequent to the application of glyphosate, the dead plants may be considered as susceptible to the herbicide and surviving plants with a substantial growth as resistant to the herbicide. A vast collection of rice germplasm resources can be screened at LD_{50} concentration for natural resistance to glyphosate and the resulting glyphosate tolerant test genotypes may be assessed for growth parameters, agronomic traits and yield penalty. Besides, status of built in endogenous herbicide resistance in elite germplasm lines may be elucidated based on quantification of shikimic acid using HPLC (Vargas et al., 2014) and using cDNA sequence variation in OsESPS gene compared to wild type rice varieties (sensitive to glyphosate). EPSPS gene-specific forward (5′-ATGGCGGCGACCATGGCGTC-3′) and reverse (5′- TCAGTTCCTGACGAAAGTG CTTAGA-3′) primers (Achary et al., 2020) can be used for the purpose using PCR analysis. Ekanyaka et al.(2016) used herbicide resistance specific E11M32 primer pair (3'-TGT AAA ACG ACG GCC AGT GAC TGC GTA CCA ATT CAC-5' and 3'-GAT GAG TCC TGA GTA AAA C-5') for AFLP (amplified fragment length polymorphism) analysis for identification of glyphosate resistance induced in rice callus.

INDUCING GLYPHOSATE RESISTANCE IN RICE

Off target movements of glyphosate causes damage to the cultivated rice by reducing the yield up to 80% (Labrada, 2007). Thus, there is a need in developing crop plants which are not affected by the broad spectrum herbicides. Inducing herbicide resistance in rice is a new means to confer selectivity and enhance crop safety and production. Differential tolerance to sub-lethal rates of glyphosate exists among rice (*Oryza sativa*) cultivars (Meier, 2011). Lakshika et al. (2019) were able to identify natural as well as induced glufosinate-resistance in selected Sri Lankan rice (*Oryza sativa* L.) varieties. Rice varieties e.g., Bg94-1, Bg403, Bg454, Bg379-2 and Ma Wee had shown the said herbicide resistance without any significant yield penalty. EMS mutated variants of these varieties even showed increased resistance to Glufosinate compared their status of natural resistance. Ekanayaka et al. (2017) developed 14 glyphosate resistant Sri Lankan rice varieties following mutagenesis with EMS (4.5 $mmol^{-1}$ for 12 h). Besides, Ekanayaka et al. (2016) reported induction of glyphosate (0.2%) resistance following in vitro mutagenesis of rice variety cv. Bg 250 with 0.1-0.2% EMS for two hours in liquid MS medium. EMS treated calli were exposed to glyphosate (0.2%) followed by test of viability using Tetrazolium test (1% Tetrazolium chloride) as per Towill and Mazur (1975). Calli that turned red indicated viable calli (glyphosate resistant) as compared to non-mutated calli which developed no colour.

Most significant level of glyphosate resistance in rice has been achieved by the process of genetic engineering which primarily focused on three strategies: overproduction of EPSP synthase, introduction of a metabolic detoxification gene and introduction of an altered EPSP synthase enzyme with decreased affinity for glyphosate (Dill, 2005). The first commercial success of glyphosate transgenics was reported in soybean in 1996 by Monsanto (Reis et al., 2006). Transgenic rice expressing a codon-modified synthetic CP4-EPSPS was reported to confer tolerance to broad-spectrum herbicide, glyphosate (Chhapekar et al., 2014). Fartyal et al. (2018) developed transgenic rice by co-expression of glyphosate tolerant mutant gene (OsmEPSPS) that over-express proline and serine; and glyphosate detoxifying genes igrA resulting in over all higher glyphosate tolerance than using either of the genes alone. However, the introduction of transgenic herbicide resistant crops in developing countries and their impacts is a controversial issue owing to various bio-safety issues.

However, it is worthwhile to focus on non-GMO approach using classical plant breeding as the herbicide resistance can be developed in rice using classical mutation breeding and in vitro mutagenesis to make stable resistant lines instead of a plant modified by genetic engineering. Once, the glyphosate resistant donors/mutants are established, traditional back cross breeding can be effectively employed for development of herbicide resistant introgression lines. Rangel et al. (2010) reported the possibility of transferring the in-built imidazolinone tolerance allele from mutant 93AS3510 to the recurrent parents 'BRS 7 Taim' and 'BRS Pelota'(Rangel et al., 2010) to develop the herbicide resistance in irrigated rice.

FUTURE PERSPECTIVE

Glyphosate resistant transgenic rice breeding lines are available, but their commercialization in India waits bio-safety clearance in terms of possibility of gene flow to non-target plants (weeds), rigorous herbicide bioassay tests and environmental pollution. However, there exists genetic variation for tolerance to glyphosate in rice especially among local land races. The natural herbicide tolerant (NHT) rice once identified, it may serve as valuable material for elucidation of in-built endogenous herbicide resistance using cDNA sequence variation. Besides, such NHT elite genotypes would pave the pathway for developing herbicide resistant high yielding breeding lines in future which eventually reduce the cost of cultivation towards weeding.

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Fluidized bed drying of food: A review

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ABSTRACT

Application of fluidized bed drying in food has been increased significantly in recent years. The fluidization technologies have been used for product development, energy efficiencies and overall quality in the final products in the area of food processing and preservation. However, this review paper evaluates different produces dried under fluidized bed dryer and to maintain the quality as well as the sensory characteristics of the dried products. This review paper suggests that fluidized bed drying certainly maintained a better drying compare to conventional drying (i.e. hot air cabinet drying) with respect to drying time, drying temperature and energy consumption of food.

Key words: Drying, fluidize bed drying, food products and quality attributes

INTRODUCTION

Drying of food products is very important because it is the easiest and the most common process of food preservation and it is one of the oldest and the most widely used methods of food preservations. Drying is indispensable unit operation at the final stage in processing of many food materials and hence, it determines, to a large extent, the quality of the product being manufactured. Drying of food is a major process operation in the food industry, consuming large quantities of energy. Dried foods are stable under ambient conditions, easy to handle, possess extended storage life and can be easily incorporated during food formulation and preparation. The drying operation is used either as a primary process for preservation, or as a secondary process in certain product manufacturing operations (Fusco et al., 1991; Senadeera et al., 2003). Longer shelf life, palatability, product diversity and substantial volume reduction are the reasons for the popularity of dried products (Chauhan and Srivastava, 2009). This could be expanded further with improvements in product quality and process application. Drying is a thermo-physical and physico-chemical operation by which the excess moisture from a product is removed. It also lowers the cost of packaging, transportation and storing by reducing both weight and volume of the final produce (Chauhan and Srivastava, 2009). The use of fluidization is one of the best technologies commonly used in drying of agro food as well as other food materials. Fluidized bed drying has been recognized as a gentle, uniform drying method and capable of drying down to very low residual moisture content with a high degree of efficiency (Borgolte and Simon. 1981). This

process is characterized by high moisture and heat transfer rates and excellent thermal control capacity compared to other conventional drying processes (Vanecek et al., 1966; Hovmand, 1987). It is also a very convenient method for drying heat sensitive food materials as it prevents them from overheating due to continuous mixing during the process (Gibert et al., 1980; Giner and Calvelo, 1987). Fluidized bed drying can be carried out as a batch or as a continuous process (Shilton and Niranjan, 1993). Fluidized bed dryers are extensively used in particular solids drying because of their high rates of heat and mass transfer and the reduced drying times. It is regarded as a fourth generation of drying process. Simultaneous heat and mass transfer processes, wherein heating medium or internal heat generation helps in evaporation of free water molecules from the product.

FLUIDIZED BED DRYING

Fluidization is widely used in processing of solid particles by various technologies such as drying, combustion, synthesis etc. Generally, fluidized bed dryers are considered superior to other conventional dryers when processing of non sticky and small size distributed particles. Once, the air is allowed to flow through a bed of solid material with a velocity greater than the settling rate of the particle, the solid particle become blown up and become suspended in the air stream. At this stage, solid particle looks like as in a boiling stage; therefore this stage is called fluidized state. Use of hot air in the fluidized bed increases the drying rate of the material (Parlak et al., 2003; Sanadeera et al., 2006; Honrvar et al., 2011). Fluidized bed contains a stainless steel chamber having a removable perforated bottom known as the bowl. The product to be dried is placed in the bowl. Air is introduced from the bottom of the bowl and is heated at a required temperature by the heaters. The various advantages in the fluidized bed are good mixing with better heat and mass transfer, higher thermal efficiency, shorter drying time and close controllable temperature as drying gas mix intensively during its passing through the fluidized bed (Kumar and Belorkar, 2015). The general process flow of a fluidized bed drying of food is illustrated herewith in Fig.1

Process flow chart of a fluidized bed drying of food

↓

Collection of raw material

↓

Sample preparation: Sorting and grading

↓

Washing and removal of water

↓

Pre treatment if required

↓

Final preparation of samples for drying

↓

Dried in fluidized bed drier with various temperature and bed height

↓

Packaging and labelling

↓

Storage

Fig. 1. The general process flow of a fluidized bed drying of food

FLUIDIZED BED DRYING APPLICATION OF FOOD

Lehmann (1992) reported that due to the high demand for encapsulated material in the global market; fluidized bed coaters have become more popular which are used for encapsulating solid or porous particles with optimal heat exchange.

Guignon et al. (2002) studied fluidized bed drying of encapsulated solid particles. They stated that the fluid bed encapsulation process consists of spraying of a coating solution into a fluidized bed of solid particles. After several cycles of wetting and drying, a continuous film is formed. Both qualitative and quantitative results are presented for several industrial applications. The main parameters affecting the process are flow-rate and pressure of the spraying liquid, composition and rheology of the coating solution, flow rate and

temperature of the fluidizing air. The rate of heat transfer between the bed and submerged surfaces is very high and the intense solid mixing inside the bed causes a temperature distribution that is almost uniform inside all the fluidized bed. These facts allow for reduction in the time of drying and level of temperature of the input hot air required for drying of the solid. Furthermore, the drying in fluidized bed avoids the formation of hot spots and makes it easy for the management of the solid as well as for the overall control of the operation (Palancar et al., 2001).

Pablo et al. (2004) investigated the drying of green peas in a fluidized bed heat pump dryer under normal and atmospheric freeze drying conditions. Three types of green peas having 8 mm and 10 mm diameter samples and two bed heights (2 and 4 mm) were used in the drying trials, operating either in isothermal conditions or on a combination of temperatures. The atmospheric freeze drying permits to obtain dried samples with high quality sensory properties. Drying kinetics was modelled with a diffusion model, and the effect of temperature on the effective diffusion coefficient follows the Arrhenius relationship. The activation energy values were 5046 and about 5910 kJ kg^{-1} for 8 mm and 10 mm diameter samples, respectively. The change in the color was negligible in the atmospheric freeze-dried samples. Additionally these dried green peas kept the original structure, yielding lower bulk density, higher values of floatability and improved rehydration ability when compared to samples dehydrated at temperature above freezing point.

Niamnuy and Sakamon, (2005) studied an industrial-scale batch fluidized bed dryer was used to dry finely chopped coconut pieces. The effects of various operating parameters i.e. the values and patterns of inlet air velocity and temperature, on the drying kinetics and some selected quality attributes of dried coconut viz. color and surface oil content were then examined. The surface oil content of the product dried by any tested conditions was still higher than that of the reference sample, which is accepted by the market.

Senadeera (2005) studied the effects of fixed bed and fluidized bed drying and their comparison on physical properties changes in spherical food materials such as peas as the model material. The physical properties such as particle density, bulk density, shrinkage and bed porosity of fresh green peas were compared in fluidized bed drying with fixed bed drying at 50°C. The results revealed that physical properties changed during both the drying processes and can be modeled with respect to the moisture content. Volume shrinkage was linearly correlated and Particle densities of peas were correlated to non-linear models. In this comparison study, lower shrinkage was experienced in fluidized bed drying compared to fixed bed drying. Low bulk density was found for the fluidized bed compared to the fixed bed. Low bulk density was also attributed to the differences in shrinkage.

Senadeera et al. (2006) investigated the changes in fluidization behaviour of green peas particulates with change in moisture content during drying under a fluidized bed dryer. All drying experiments were conducted at 50 ± 2°C and 13 ± 2 % RH using a heat pump dehumidifier system. Fluidization experiments were undertaken for the bed heights of 100, 80, 60 and 40 mm and at 10 per cent moisture content levels. Fluidization behaviour was best fitted to the linear model of Umf = A + B m. where A, B are constants, U= Fluidization velocity, mf= Minimum fluidization and m= moisture content. A generalized model was also formulated using the height variation and which was used to compare minimum fluidization velocity with Ergun equation. The results revealed that there is a continuous change in the dimensions of the food particulates during fluidized bed drying resulting changes in minimum fluidization velocity. Change in minimum fluidization velocity was linear with the reduction of moisture content for spherical particulate (peas). It was also found that, the Generalized model and Ergun model can be used to predict minimum fluidization velocity with reasonable accuracy due to the spherical nature of the product shape.

Zhanyong et al. (2006) developed a modified fluidized bed termed as pulsed fluidized bed (PFB), to eliminate some limitations of the conventional fluidized bed (40, 50, 60, 70 and 80°C) by superposing a pulsating air stream (120-320 $m^3 h^{-1}$) with a desirable air temperature (80 and 90°C) on the continuously flowing fluidizing air. The PFB drying of green peas is superior to that in FB in terms of drying rates as well as colour preservation. The drying techniques are best suited for seat sensible agricultural products. Due to good dispersion of solids by the pulsing air flow, a higher drying rate is realized in PFB with lesser colour degradation and reduction in shrinkage of green peas.

Jingsheng et al. (2008) studied on soybean seeds which were contacted with silica gel in a fluidized bed, where the mass transfer was driven by moisture concentration gradient. It has the better advantage of well-mixing the solid adsorbent (silica gel) with the material being dried (soybean seeds) in fluidization state, and thus the dried seeds quality could be improved since they are in a uniform environment of low humidity. Separation of these two solids once desired moisture content is reached can be realized due to the size difference as well as the operation conditions of fluidization. The drying kinetics was compared under different mass ratios and fluidization conditions. The dispersion rate of soybean seeds was increased with addition of either silica gel particles, whereas the drying rate was improved with the gas velocity and mass ratio of silica gel. A scheme of sorption drying in a hybrid process of fluidized/fixed bed is proposed in the viewpoint of energy efficiency and product quality.

Singh et al., (2008) studied drying of ginger flakes under fluidized bed conditions in the air temperature ranges from 50 to 80°C and pretreatment of calcium oxide in the range of 1 to 2.5 per cent . They reported that drying rate at 60°C air temperature decreased from 0.43 to 0.17 g s^{-1} with the change in moisture content from 300 to 10 per cent (d.b.). The volatile oil in dried in dried ginger flakes decreases from 1.2 to 0.99 per cent with increase in drying temperature from 50 to 80 °C.

Srinivasakannan and Chandrasekhar (2008) reported fluidized bed drying of mustard seeds. Mustard is one of the popular oil seeds which were investigated for drying in batch fluidized beds with fluidization column of 0.245 m internal diameter and a height of 0.6 m.. Experiments were conducted to assess the kinetics of drying for the variation in the inlet air temperature of 60, 70 and 80 °C, the inlet air flow rate and the solids holdup in the fluidized bed. The drying rate was found to increase significantly with increase in temperature and with flow rate of the heating medium, while decrease with increase in solids holdup. The duration of constant rate period was found to be insignificant, considering the total duration of drying. The drying rate was compared with various exponential time decay models and the model parameters were evaluated. The page model was found to match the experimental data very closely with the maximum root mean square error (RMSE) of less the 2.0%. The experimental data were also modeled using Fick's diffusion equation and the effective diffusivity coefficients was found to be within 1.69 × 10-11 to 3.26 × 10-11 $m^2 s^{-1}$ for the range of experimental data covered in the present study with RMSE less than 4%.

The variation of shrinkage and moisture diffusivity with temperature and moisture content for green peas under pilot scaled fluidized bed dryer (FBD) with inert particles assisted by an infra red (IR) heat source were studied by Honarvar et al. (2011). The shrinkage was only a function of moisture content; the moisture diffusivity was dependent upon both temperature and moisture content. The effective diffusion coefficients were evaluated in a temperature range of 35-70 °C and a moisture content range of 0.25 - 3.8 kg moisture per kg dry solids.

Deshmukh et al. (2015) assessed the drying characteristics of materials (pulses granules) in the fluidized bed dryer in drying temperature of 80 to 100°C with respect to the various operating variables and distributor plates. The drying rate increased as increasing the velocity of the drying air, while decreased with increases solids holdup. The drying rate was found to increase significantly with increase in temperature and flow rate of the

heating medium as the time increases. Best result were found in case for moisture ratio and initial to the final moisture content is split pea gram and split red gram is found in case of mass in dry basis and wet basis. The drying rate was found to increase significantly with increase in temperature and flow rate of the heating medium as the time increases. Best results were found in case for moisture ratio and initial to the final moisture content in case of split pea gram. In case of split red gram, mass in dry basis and wet basis were found to be best. Many researchers round the globe have studied on fluidized bed drying and their major findings are presented in Table 1.

Table 1. Research findings on fluidized bed drying

Researchers	Findings
Borgolte and Simon (1981)	The energy required to accomplish this task is huge, and much work has been devoted to maximizing the thermal efficiency of dryers in order to reduce the necessary heat consumption. Fluid bed drying has been recognized as a smooth, uniform drying method, capable of drying down to very low residual moisture content with a high degree of efficiency. This process is characterized by high moisture and heat transfer rates and excellent thermal control capacity compared with conventional drying processes (Vanecek et al., 1966; Hovmand, 1987).
Sokhansanj and Jayas (2006)	Fluidized bed drying is a well-known process that has been widely used in the dairy and pharmaceutical industries for drying, granulating, and coating operations. Fluidized bed drying process has been successfully used to dry many agricultural products of different particle sizes (ranging from 10 mm to 20 mm) such as wheat and corn grains; cut green beans; slices of carrot, celery, mango, or kiwifruit; button mushrooms; and green peas. In fluidized bed drying, the drying air is introduced at a velocity at which the material remains fully suspended in the hot air stream and dries with high rates of heat and moisture transfer.
Thakur and Gupta (2007)	Paddy grains subjected to fluidized bed drying intervening rest duration between first and second stage of drying, enhanced drying rate, reduced energy consumption and improved head rice yield. Energy requirement can be significantly saved (9–58 %) by providing rest durations (30–120 min) in comparison to the continuous drying.
Murthy and Joshi (2007)	Sun drying required the longest period of drying 660 min (11 hrs), while the shortest time of drying is with fluidized bed drying at 80°C with 115 m min^{-1} air velocity 120 min (2 hrs). The results indicate that there is great loss of most of the ascorbic acid in the aonla slices. The retention of ascorbic acid in the samples dried in fluidized bed drying is greater compared to those dried under sun and hot air tray.
Herguido et al. (1992)	Sawdust drying was studied; the gasification of sawdust in a fluidized bed indicate moisture content need to be 8-12 %. The pulsed fluidized bed dryer is a modified conventional fluidized bed in which gas pulses cause vibration of the particle bed.

CONCLUSION

This review concludes the importance of fluidized bed drying of food. The application of fluidized bed dryer in industries has gain importance because of increase in energy and operational efficiencies. Fluidization gives higher benefits as compare to traditional method as it provides maximum surface area for higher heat and mass transfer rates during drying.

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Integrated nutrient management practices for sustainable soybean [*Glycine max* (L.) Merr.] production: A case study of Ghazni province of Afghanistan

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ABSTRACT

A field experiment was carried out during spring season of 2020 at Ghazni province, Afghanistan to evaluate the effects of integrated nutrient management on growth and productivity of soybean and to develop a suitable fertilizer package of integration of inorganic fertilizers with biological and organic manures for soybean. The experiment was laid out in Randomized Block Design (RBD) with three replications having seven treatments. T_1= Absolute control, T_2 =100% RDF, T_3 = 75% RDF + FYM (5 t ha^{-1}), T_4 = 75% RDF + BF (Rhizobium + PSB), T_5 = 50% RDF + FYM (10 t ha^{-1}), T_6 = 50% RDF + BF (Rhizobium + PSB), T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB). Perusal of the results revealed that application of 50% RDF + FYM (5 t ha^{-1}) + BF(Rhizobium + PSB) recorded significantly higher plant height, number of branches, number of root nodules, number of pods per plant (82.7), number of seeds per pod (2.6), 100 seeds weight (12.5 g), seed yield (2687.5 kg ha^{-1}) and straw yield (4424.8 kg ha^{-1}) and gross return (196,977 Afn. ha^{-1}) over control and treatment that received 50% RDF + BF(Rhizobium + PSB). Although, the maximum net return (145,801 Afn. ha^{-1}) was obtained with 100% RDF, but it was non-significant with the application of 50% RDF + BF+ 5t FYM ha^{-1}(143,227 Afn. ha^{-1}). Based on the result of experiment it is recommended that for remunerative soybean cultivation and soil health, the farmer should apply 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB).

Key words: Biofertilizer, FYM, integrated nutrient management, soybean

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a species of legume native to East Asia. The plant is classified as an oilseed as well as pulse. It is an annual plant that has been used in China for 5000 years as a food and a component of drugs. Soybeans are important source of vegetable oil and protein worldwide. Together, oil and protein content account for about 60% of dry soybean by weight. Protein at 40% and oil at 20%, remainder consist of 35% carbohydrate and about 5 per cent ash. It is also called as "gold of soil" due to atmospheric N fixation in soil to maintain the soil fertility and beneficial effect on following crop (Kausadikar et al., 2003).

Soybean accounts for about 50% of oilseeds production in the world. The protein form of soybean is equivalent in quality to that of meat, milk products and eggs. The soya meal is an important human food and soya flour is essential in the various preparations viz. bread, cakes, muffins, biscuits and pastry. Worldwide the cultivation area for soybean is accounted 122.30 million hector with average productivity of 2.70 t ha^{-1} and 330.41 million metric tons production (USDA, 2016). In Afghanistan soybean consumption is going rapidly and is used as soy naan, soy dishes, including soybean korma (soup), soymilk and full-fat soy flour. Production of soybean was recorded 4,000

MT with average productivity of 0.6 to 1.2 t ha^{-1} (NEI, 2015; NEI, 2011). In spite of high yield potential, soybean productivity is much less in Afghanistan than the world average. Among the factors responsible for low productivity, inadequate fertilizer use and emergence of multi-nutrient deficiencies due to poor recycling or organic sources and unbalanced use of fertilizers particularly micronutrients (Chaturvedi et al., 2010). One of the major constraints for low productivity of soybean is inadequate supply of nutrients in balanced manner. It has been established that continuous use of high analysis chemical fertilizers leads to deficiency of secondary and micronutrients, soil salinity and environmental pollution. While Afghan farmers are confined to use high chemical fertilizers which supply only the major nutrients while the crop plants remove all the essential nutrients, which are needed for their normal growth. Mention the recommended dose of fertilizer for Afghanistan and farmers' practice. The chemical fertilizers are also responsible for creating heavy metal pollution in soil. The heavy metal considered to be a threat to the environment are Arsenic (As), Cadmium (Cd), Copper (Cu), Mercury (Hg) and Lead (Pb). The soil factors which are affected by heavy metals are pH, clay content, texture, organic matter content, phosphate additions and exchangeable sodium percent (Singh et al., 1979). Continuous use of chemical fertilizer is leading reduction in crop yield and resulted in imbalance of nutrients in soil, which has adverse effects on soil health. Use of organic manure alone or in combination with chemical fertilizers will help to improve physio-chemical properties of the soil, efficient utilization of applied fertilizers for improving seed yield and quality. The appropriate combination of mineral fertilizers with organic manure can be feasible to sustain agriculture as commercial and profitable ensuring high yield of crop without deterioration in quantity and quality of the produce and soil health.

There seems a wide potential to upgrade efficiency of these nutrients through better agronomic practices. The low practical options to upgrade the efficiency of applied fertilizer nutrients are judicious combinations of organic and inorganic sources and promoting the use of bio-fertilizers. In recent years, a concept of integrated nutrient supply involving use of organic manures and inorganic fertilizers has been developed to obtain sustained agricultural production (Gaikwad et. al, 1996). Integration of organic and inorganic sources of nutrients along with bio-fertilizers is found to give higher productivity and monetary returns in soybean (Singh and Rai, 2004; Bhattacharyya et al., 2008). Integration of chemical fertilizer with organic manures has been found to be quite promising not only in maintaining higher productivity but also providing greater stability in crop production (Nambiar and Abrol, 1992). Further the organic sources unlike inorganic ones have substantial residual effect on succeeding crops (Shivakumar and Ahlawat, 2008). Whereas integrated nutrient management (INM) involves the use of manures, bio-fertilizers and chemical fertilizers to achieve sustained crop production and maintain better soil health. INM is best approach for better utilization of resources and to produce crops with less expenditure.

Hence, integrating the use of organic input, inorganic input and Bio-fertilizers (Rhizobium+ PSB) would improve the productivity of crop on sustainable basis. Therefore, the present study was aimed to evaluate the effects of integrated nutrient management on growth and productivity of soybean and to develop a suitable fertilizer package by integrating inorganic fertilizers with biological and organic manures for soybean.

MATERIALS AND METHODS

The present investigation was conducted in Mohammad Khan village, Ghazni, Afghanistan, during spring season of 2020. The experimental field is located at longitude 68° 20' 11" East and latitude 33°31' 48" North at an elevation of 2204 m above mean sea level. The Ghazni province is located in the southeast region of Afghanistan. It acquires a transitional climate between semi-arid with cold winter and warm dry summer. Total precipitation is 250 – 300 mm and mostly occurs in winter in the form of snow. The soil of experimental field was sandy clay loam in texture, medium in organic matter and available N was low (233 kg ha^{-1}), P was medium (15.9 kg ha^{-1}) and K was high (392 kg ha^{-1}).

Therefore, the recommended doses of fertilizer (RDF) were maintained on the basis of initial status of available nutrients in the experimental soil to carry out the present investigation.

Experimental design and treatments

The experiment was laid out in Randomized Block Design (RBD) with three replications having seven treatments. T_1= Absolute control, T_2 =100% RDF, T_3 = 75% RDF + FYM (5 t ha^{-1}), T_4 = 75% RDF + BF (Rhizobium + PSB) (5g culture each of Rhizobium and PSB per kg Seed), T_5 = 50% RDF + FYM (10 t ha^{-1}), T_6 = 50% RDF + BF (Rhizobium + PSB) (5g culture each of Rhizobium and PSB per kg Seed), T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) (5g culture each of Rhizobium and PSB per kg Seed). Calculated quantity of fertilizers and farm yard manure were applied plot wise as per treatments. Fertilizer doses were calculated on the basis of recommended dose of nutrients 20 kg N + 60 kg P_2O_5 + 20 kg K_2O + 5 kg Zn ha^{-1} through urea, triple superphosphate, sulphate of potash, and zinc through zinc sulphate respectively. All the fertilizers were given as basal dose. Well-decomposed farm yard manure as per treatments was applied before sowing and incorporated in the soil. Recommended package of practices were followed uniformly for all the treatments.

Data collection and analysis

Five plants were selected randomly from each plot and tagged for recording plant height, branches per plant, plant dry weight, per pods plant and seeds per pod. For root nodule count, plants were uprooted carefully with the help of khurpa (hoe), the roots were washed carefully and nodules from the tap and lateral roots were counted and the average values were recorded. For yield estimation, the crop was harvested from the net plot area and the pods were threshed manually. The weight of cleaned seeds was recorded as net plot yield. The dried Stover from each plot was harvested and weighed. Both seed and straw yield were expressed as kg ha^{-1}. The harvest index (HI) was computed by dividing grain yield with total biological yield. For computing of benefit:cost ratio, the net return was divided with the cost of cultivation. The value so obtained was considered as cost benefit ratio.

BCR= Net Return/ Total cost of production

Data were analyzed using ANOVA for randomized block design (Rana et al., 2014). Significance of difference among different treatments was tested using F-test. Critical difference (CD) values were calculated for the parameters that exhibited significant differences. The treatment means were compared at 5 per cent level of significance.

RESULTS AND DISCUSSION

Influence on growth

At successive growth stages plant height was significantly influenced by the application of inorganic fertilizers, bio-fertilizer and farm yard manure. At 30, 60 and 90 DAS, significantly higher plant height was recorded with application of 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) over control treatment and application of 75% RDF + BF and 50% RDF + BF (Rhizobium + PSB), but was at par with rest of the treatments (Table 1). It was observed that the integration of bio-fertilizer and organic manure with chemical fertilizers had a significant impact on plant height. This clearly indicated the need for adding organic manures to the soil conjunctive with inorganic fertilizers, which increased the availability of nutrients considerably resulting in a positive effect on growth parameters. These findings are in accordance with the results of Babalad (1999) who had observed increased plant height, number of trifoliate leaves per plant and number of branches per plant in soybean due to the application of organic manure and inorganic fertilizers. Similar findings were reported by Babhulkar et al. (2000), Jayabal et al., 2000, Dikshit and Khatik (2002) in soybean, sunflower and sorghum.

Number of branches per plant differed significantly, as influenced by different nutrient management treatments, at all stages of crop growth (Table 1). At 30, 60 and 90 DAS, application of 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) recorded significantly higher number of

branches over control treatment and the treatment which received 50% RDF with only bio-fertilizer, but it was found on par with other treatments. The increase in plant growth attributed to the increase availability of nutrients with the application of inorganic fertilizer, continuous supply of macro and micro nutrients from FYM, which helped in acceleration of various metabolic processes viz. photosynthesis, energy transfer reaction and symbiotic biological N - fixation process. These results are in close agreement with the findings of Bhaskar (2013) who reported that the number of branches per plant was significantly higher with the application of 75% RDF + Zinc (5 kg ha^{-1})+BF (Rhizobium+ PSB)+FYM (2.5 t ha^{-1}) over absolute control but it was at par with 50% RDF+Zinc (5 kg ha^{-1})+BF (Rhizobium +PSB)+FYM 5 t ha^{-1}. The effect of integrated nutrient management at all growth stages on number of root nodules per plant was significant. At 30, 60 and 90 DAS stages application of 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) recorded the highest number of root nodules per plant (12.2, 17.4 and 24.7, respectively), which were significantly superior to the absolute control and the treatment that received only 50% RDF + BF (Rhizobium + PSB), but it was at per with rest of the treatments. The increase in nodules number is the response of inoculation by bio-fertilizer which might have accelerated the nitrogenous activity in plant system and hence such response. Nodules are the niches of microorganisms and fixes atmospheric nitrogen. Also higher number of root nodules per plant might be due to increased nutrients availability, which resulted in the formation of active and more number of root nodules. The results are in close agreement with the findings of Lone et al. (2009), Mohod et al. (2010) and Bhaskar (2013) who reported that the maximum number and dry weight of root nodules per plant were recorded with the application of 75% RDF+Zinc (5 kg ha^{-1})+BF (Rhizobium+ PSB)+FYM (2.5 t ha^{-1}).

Table 1. Effect of integrated nutrient management on growth parameters of soybean

Treatment	Plant height (cm)			Number of branch per plant			Number of root nodules per plant		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1= Absolute control	12.1	41.7	63.2	2.0	2.7	3.9	7.1	11.3	14.9
T_2 =100% RDF	16.2	51.3	79.9	2.7	4.0	5.1	11.6	16.2	23.3
T_3 = 75% RDF + FYM (5 t ha^{-1})	14.8	49.7	75.1	2.6	3.8	4.9	10.8	15.5	22.5
T_4 = 75% RDF + BF (Rhizobium + PSB)	14.8	46.7	73.0	2.4	3.7	4.8	10.5	14.8	21.7
T_5 = 50% RDF + FYM (10 t ha^{-1})	15.3	54.7	83.8	2.7	4.1	5.3	11.9	16.8	24.1
T_6 = 50% RDF + BF (Rhizobium + PSB)	14.2	43.2	68.7	2.2	3.1	4.6	9.5	13.0	19.2
T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB)	16.8	54.9	84.0	2.9	4.2	5.4	12.2	17.4	24.7
SEm (±)	0.87	2.51	2.96	0.17	0.22	0.24	0.55	0.90	1.14
CD (P=0.05)	2.67	7.732	9.119	0.513	0.67	0.75	1.71	2.76	3.50

Influence on yield and yield attributes

The yield characteristics viz., numbers of pod per plant, seeds per pod and 100-grains weight were found to vary significantly among the different treatments as influenced by integrated nutrient management practices. Maximum number of Pods per plant (82.7) were recorded by treatment which received 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) than absolute control and the treatment which received only 75% RDF + BF (Rhizobium + PSB), but it was statistically on per with other treatments. Also application of BF (Rhizobium + PSB) recorded significantly higher number of seeds per pod (2.6) and 100-grain weight (12.5 g) as compare to control treatment (Table 2). Improvement in yield components might have resulted from favorable influence of integrated nutrient management practices on the growth characteristic viz., plant height, branches per plant and root nodule account and efficient and greater partitioning of metabolites and adequate translocation of nutrients to developing reproductive structures. Similar findings were also recorded by Singh and Rai (2004), they reported that the highest pods per plant, seeds per pods and 100-grain weight of soybean were recorded through the combination of recommended dose of NPK + FYM @ 5 t ha^{-1} + bio-fertilizers. The results of the investigation showed that different treatments significantly influenced the grain yield of soybean. Application of 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) recorded maximum grain yield (2687.5 kg ha^{-1}), which was significantly higher than absolute control and T6 = 50% RDF + BF (Rhizobium + PSB), but it was at par with other treatments (Table 2).

Table 2. Effect of integrated nutrient management on yield attribute and yield of soybean

Treatment	Pod per plant	Seed per pod	100-grain weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)
T_1= Absolute control	62.3	2.0	10.6	1545.6	2555.8	37.7
T_2 =100% RDF	80.7	2.5	12.3	2577.9	4042.8	39.0
T_3 = 75% RDF + FYM (5 t ha^{-1})	78.9	2.4	11.6	2530.3	4019.3	38.7
T_4 = 75% RDF + BF (Rhizobium + PSB)	74.7	2.4	11.6	2503.1	3919.1	39.0
T_5 = 50% RDF + FYM (10 t ha^{-1})	81.7	2.5	12.4	2598.6	4077.1	39.0
T_6 = 50% RDF + BF (Rhizobium + PSB)	69.9	2.3	11.6	2311.7	3547.5	39.5
T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB)	82.7	2.6	12.5	2687.5	4424.8	38.0
SEm (±)	3.15	0.12	0.38	97.10	202.63	0.44
CD (P=0.05)	9.72	0.36	1.17	299.20	624.368	ns

Similar finding was recorded by Koushal and Singh (2011) who observed the highest seed yield in the treatment that received 50% recommended N applied through urea + 50% N applied through FYM + PSB and the lowest seed yield in control. Kumpawat (2010) also reported that integrated nutrient management of inorganic chemical fertilizers along with application of FYM and inoculation with biofertilizers (rhizobium and PSB) produced higher yield. Straw yield also showed significant differences due to different treatments. Higher straw yield (4424.8 kg ha^{-1}) was recorded with the T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) as compared to absolute control and treatment that received 75% RDF+ BF (Rhizobium + PSB) (Table 2). Increasing in straw yield in this treatment occurred mainly due to maximum plant height and higher number of branches per plant. Such finding was supported with the work of Koushal and Singh (2011) thy reported that the highest straw yield was recorded in the treatment 50 % recommended N applied through urea+50% N applied through FYM+PSB. Menaria et al. (2003) reported that application of 75% recommended N through RDF+25% N through weed biomass + PSB + Rhizobium increased growth and yield contributing parameters. Harvest index is the ratio of grain yield to biological yield. The result of this study showed that there were non-significant differences among different treatments for harvest index (Table 2). Data are mentioned in table and hence only significant major finding should be highlighted in the text not big paragraphs on individual characters. Characters having similar trends should be combined and mentioned in short.

Economics

Economics of production is very important aspect to a judge the efficiency of different production system based on physical feasibility and its commercial viability, economics determination, cost of cultivation, gross returns, net returns and B: C ratio were considered. The compatibility of different treatments was workout with a view to understanding its feasibility and economic viability in a given system. In the current study significantly higher gross return (196,977 Afn. ha^{-1}) of soybean was recorded with Application of 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) compared to control treatment and the treatment that received 50% RDF + BF (Rhizobium + PSB) and it was on par with the rest of the treatments (Table 3). The higher gross return was mainly due to higher grain and straw yields of soybean in the respective treatments. These results are in close agreement with the findings of Farhad et al. (2017) who reported that the maximum gross return and gross margin were recorded from the treatment T6 which was 50% recommended dose (N-P-K) + 1.2 kg ha^{-1} biofertilizer. The lowest gross return and gross margin were obtained from the control treatment. Net return is actual profit gained under a particular treatment by subtracting the cost of cultivation from the gross return under the same treatment. The maximum net return (145,801 Afn. ha^{-1}), was obtained with 100% RDF, it was followed by the 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB), 75% RDF + BF (Rhizobium + PSB), 75% RDF + FYM (5 t ha^{-1}), 50% RDF + BF (Rhizobium + PSB) and 50% RDF + FYM (10 t ha^{-1}). That they were all significantly higher than absolute control, but they was at par to each other (Table 3). Production of higher net return by application of 100 RDF and other treatments could be attributed to higher grain yield of soybean and reduction in cost of cultivation. Significantly higher B:C ratio (3.41) was recorded with the application of 100 % RDF over absolute control, 75% RDF + FYM (5 t ha^{-1}), 50% RDF + FYM (10 t ha^{-1}) and 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB), but it was at par with the application of 75% RDF + BF (Rhizobium + PSB) and 50% RDF + BF (Rhizobium + PSB). Application of 50% RDF + FYM (10 t ha^{-1}) recorded significantly lower B:C ratio (1.87) over all other treatments (Table 3). Production of lower B:C ratio by application 50% RDF + FYM (10 t ha^{-1}) attributed to higher cost of cultivation, which consequently helped in recording significantly lower B:C ratio.

Table 3. Effect of integrated nutrient management on gross returns, net returns and B:C ratio of soybean

Treatment	Gross return (Afn. ha^{-1})	Net return (Afn. ha^{-1})	B:C ratio
T_1= Absolute control	113,306	83,543	2.81
T_2 =100% RDF	188,539	145,801	3.41
T_3 = 75% RDF + FYM (5 t ha^{-1})	185,162	130,668	2.40
T_4 = 75% RDF + BF (Rhizobium + PSB)	183,055	141,061	3.36
T_5 = 50% RDF + FYM (10 t ha^{-1})	190,058	123,808	1.87
T_6 = 50% RDF + BF (Rhizobium + PSB)	168,912	130,161	3.36
T_7 = 50% RDF + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB)	196,977	143,227	2.66
SEm (±)	7,167.3	7,167.3	0.147
CD (P=0.05)	22,084.5	22,084.5	0.454

*One USD is equal to with 77 Afn.

CONCLUSION

After going through the finding of the present study, it can be concluded that integrated nutrient management of 50% recommended dose of chemical fertilizers + FYM (5 t ha^{-1}) + BF (Rhizobium + PSB) was more effective compared to other treatment combinations in recording higher growth parameters, seed yield and net return of soybean.

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Response of hybrid maize to application of nutrients in laterite soil

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ABSTRACT

A field experiment was conducted during *kharif* 2019 at the Instructional Livestock Farm, of OUAT, Bhubaneswar, India, to study the response of hybrid maize to application of nutrients in laterite soil. The hybrid maize variety Kalinga Raj (OMH 14-27) was grown for the experiment. There were eight treatments comprising nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) such as T_1-120:60:60 kg ha^{-1},T_2-120:60:60 kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1},T_3-150:75:75 kg ha^{-1},T_4-150:75:75 kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}, T_5-180:90:90 kg ha^{-1}, T_6-180:90:90 kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1}+Borax @10 kg ha^{-1},T_7-STBFR (150:39:45 kg N:P_2O_5:K_2O ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1} and T_8- STCR (216: 15: 21 kg N:P_2O_5:K_2O ha^{-1}) laid out in Randomized Block Design with three replications. Results revealed that, T_7 – Soil Test Based Fertilizer Recommendation (STBFR) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} recorded maximum grain yield (8256 kg ha^{-1}) and stover yield (10353 kg ha^{-1}) followed by treatment T_8- Soil Test Crop Response (STCR) based fertilizer application. Minimum grain yield (6737 kg ha^{-1}) and stover yield (9644 kg ha^{-1}) were recorded in treatment of T_1, the conventional farmers' practice is being followed in Odisha since long. The treatment T_7 also recorded the most effective plant growth parameters such as plant height (212.87 cm) and number of green leaves per plant (11.73), leaf area index (2.26), dry matter accumulation (1423.76 g m^{-2}) and crop growth rate (6.54 g m^{-2} day $^{-1}$) at the time of harvest, number of days to 50% tasseling (56.33), number of days to 50% silking (58.00) followed by T_8 which was at par with T_7. T_7 treatment also recorded significantly maximum yield attributes such as cob length (18.06 cm), cob girth (15.94 cm), number of rows per cob (15.2), number of grains per row (32.13), 1000 grain weight (282.74 g) followed by T_8. Cost of cultivation was Rs. 36000 ha^{-1} in T_1. T_7 fetched the maximum gross return (Rs.141678 ha^{-1}) and net return (Rs. 99178 ha^{-1}) with benefit: cost ratio of 3.33 which was found to be highest as compared to other approaches of nutrient management practices.

Key words: Growth parameters, hybrid maize, STBFR, STCR fertilizer application, yield attributes

INTRODUCTION

Maize (*Zea mays* L.) is the 3rd most important food crop after rice and wheat in world. Due to its photoperiod insensitivity, it can be grown throughout the year under varied climatic conditions. It has very high genetic yield potential because of its C_4 pathway, is known as the "Queen of cereals". The average *kharif* maize productivity of Odisha is higher (2098 kg ha^{-1}) than the national average productivity (2015 kg ha^{-1}). Maize is being cultivated in an area of 2.52 lakh hectares with a production of 7.45 lakh tones and productivity of

2098 kg ha^{-1} (Anon., 2016-17). The *kharif* maize contributes to the tune of 92 % to the total maize production. It is rich in carbohydrates, protein and fairly good source of calcium, phosphorus, iron, and vitamin-A, nicotinic acid and riboflavin (Kumar et al., 2016). Being an exhaustive crop, it's yield level depends on nutrient supplying capacity of the soil and requires both micro and macro nutrients in adequate quantity to obtain high growth and yield (Ghosh and Anchal, 2019). The cultivation of maize in Odisha is dependent on monsoon rainfall and nutrient removal is far excess of their replenishment under intensive cropping led to widespread multi nutrient deficiency related low productivity of crop. Fertilizer management is one of the most important factor that influence the growth and yield of maize crop. Soil test based application of plant nutrient helps to realize higher response and benefit: cost ratio as the nutrients are applied in proportion to the magnitude of the deficiency of a plant nutrient and its correlation with the nutrients imbalance in soil, helps to harness the synergistic effects of balanced fertilization (Rao and Srivastav, 2000). STCR targeted yield approach has been found to be beneficial which recommends balanced fertilization considering the soil available nutrient status and crop needs. Keeping in view the above facts, the experiment was undertaken to assess the performance of hybrid maize (var. Kalinga Raj) under various nutrient management practices in laterite soil of Bhubaneswar.

MATERIALS AND METHODS

The study was undertaken at the Instructional Livestock Farm of Odisha University of Agriculture and Technology, Bhubaneswar (20°17'N, 85 47 E, 25.9 m above mean sea level) during *kharif* season of 2019 with hybrid maize variety Kalinga Raj (OMH 14-27).The soil of the experimental site is a well drained upland land with loamy sand in texture. The pH (1:2.5, soil: water suspension), EC, soil organic carbon, available nitrogen, phosphorus, potassium, sulphur, zinc and boron content of the experimental soil were found to be 5.15, 0.65 dS m^{-1}, 0.48%, 162.5 kg ha^{-1}, 100.6 kg ha^{-1}, 413 kg ha^{-1}, 16.66 kg ha^{-1}, 14.08 kg ha^{-1} and 2.158 kg ha^{-1}, respectively. The experiment was conducted with eight treatments (T_1-120:60:60 kg N:P_2O_5:K2O kg ha^{-1}, T_2 120: 60: 60 kg N:P_2O_5: K_2O kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}, T_3-150:75:75 kg N: P_2O_5: K_2O ha^{-1}, T_4- 150:75:75 kg N: P_2O_5: K_2O ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}, T_5-180:90:90: N:P_2O_5: K_2O kg ha^{-1}, T_6-180:90:90:N:P_2O_5: K_2O kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}, T_7- STBFR (150:39:45 kg N:P_2O_5: K_2O ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} and T_8-STCR (216:15:21 N: P_2O_5: K_2O kg ha^{-1}) laid out in Randomized Block Design with three replications .The recommended dose of NPK was 120-60-60 kg N: P_2O_5: K_2O kg ha^{-1}. A uniform dose of 10 t ha^{-1} well decomposed FYM was applied during final land preparation and mixed thoroughly with soil. The chemical sources of fertilizers used for N, P and K were Urea, DAP and MOP and these were applied for the treatments. For Zn 25 kg ha^{-1} Zn SO_4 and for B borax @ 10 kg ha^{-1} were applied as per the treatments. Full dose of Zn, B, P_2O_5, K_2O and 1/3rd of N were applied as basal. The second split of N (1/3rd of total N requirement) was top dressed at knee high stage and third split of rest 1/3rd N was applied at tasseling stage. In T_7 macro nutrients were applied on the basis of Soil Test Based Fertilizer Recommendation (STBFR). In T_8 it was on the basis of Soil Test Crop Response (STCR). Soil Test Crop Response (STCR) approach is a comprehensive approach of fertilizer utilization, where in fertilizer would be applied based on the yield target, site specification, crop specification and soil test values. This equation includes parameter like:

i. Nutrient requirement (NR) kg q^{-1} of economic product,

ii. Efficiency of soil estimated nutrient (%) =Cs,

iii. Efficiency of organic matter (%) and

iv. Efficiency of fertilizer nutrient (%) =Cf

$$\text{FD (kg ha}^{-1}) = \frac{\text{NR X 100 X T}}{\text{Cf}} - \frac{\text{Cs}}{\text{Cf}} \text{ X SN or SP or SK}$$

T= Target yield in q ha^{-1}, S= soil estimated nutrient kg ha^{-1}

Here, T= 8t ha^{-1}= 80 q ha^{-1}

FN= 3.96 x T – 0.62 SN, FP_2O_3= 1.56 x T – 1.93 $SP_2O_5FK_20$= 1.66 x T – 0.27 K_2O

Hence, fertilizer dose required = 216: 15: 21 kg N: P_2O_5: K_2O kg ha^{-1}

Biometric observations were taken following standard procedure. The economics was calculated and statistical analysis of data was done following standard formulae.

RESULTS AND DISCUSSION

Data presented in Table 1 reveals that, significantly taller plants at harvest were recorded with T_7-STBFR+ $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1} followed by T_8-STCR, where as the minimum plant height was observed in T_1 due to low nutrient level. A significant and positive correlation between fertilizer dose and plant height was also observed by Kumar et al. (2007).

Significantly maximum number of leaves per plant and LAI were recorded in T_7- STBFR + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} .This might be due to balanced nutrient management with primary, secondary and micronutrients. The LAI determines the dry matter production in the plant in influencing the photosynthetic efficiency. Application of STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} in T_7 resulted in more vigorous crop growth at all phases of growth and produced maximum dry matter followed by T_8. Significantly minimum dry matter was produced in the treatment which received only 120:60:60 kg N: P_2O_5: K_2O ha^{-1} (T_1). The LAI, dry matter accumulation in T_7 were 1.29 and 1.15 per cent higher respectively than that recorded in T_1. This was in agreement with the findings of Arya and Singh (2000) and Kumar et al. (2007). More dry matter with the application of STBFR (150:39:45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @25 kg ha^{-1} + Borax @10 kg ha^{-1} (T_7) over other nutrient management practices is attributed to more number of green leaves per plant, plant height, leaf area index and dry matter production.

The results pertaining to number of days taken to 50 per cent flowering (i.e. silking in female parent and tasseling in male parent) of maize hybrid as influenced by the nutrient management practices are furnished in Table 2. The significant difference was observed on days to 50 per cent silking and tasseling in female and male parents of maize hybrid respectively due to the effect of different treatment of fertilizer applications. The female parent took maximum number of days to 50 per cent silking of 58.00 days with the application of STBFR (150:39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1} in T_7 followed by 57.33 days with the application of STCR (216:15:21 kg N: P_2O_5: K_2O ha^{-1}) in T_8. Hence, treatment T_7 was observed to be significantly superior to other treatments. Similarly, the male parent of hybrid maize took highest mean number of days to 50 per cent tasseling of 56.33 days in T_7 followed by T_8. Whereas significantly minimum number of 53.33 days were taken for 50 per cent tasseling by the male parent in T_1.

The dry matter production during post anthesis period depends upon potential ability of the plant to photosynthesize (source capacity and intensity) and the capacity of the grain (sink capacity and intensity) to accept the photosynthates. The sink is composed of cob length, cob girth, number of rows per cob, number of grains per row and grain weight. All these components have been found to be markedly influenced by the nutrient approaches applied during the period of study. Application of STBFR (150:39:45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} in T_7 recorded significantly maximum cob length (18.06 cm), cob girth (15.94 cm), number of rows per cob (15.2), number of grains per row (32.13) with bolder grains (thousand grain wt-282.74 g) followed by T_8-STCR (216: 15:21 kg N: P_2O_5: K_2O ha^{-1}). The number of grains per row and thousand grain weight in T_7 and T_8 were at par. Increased length of cobs with increased fertilizer application could be attributed to the increased physiological processes leading to higher growth and increased transport of photosynthates to sinks. Similar trend was noticed in the cob girth as that of cob length. This was due to higher rate of fertilizer application which enhanced photosynthetic rate. Similar results were reported by Arun Kumar et al. (2007).The number of rows per cob also followed same trend as that of girth and length of cob. In T_8-STCR (216:15: 21 kg N: P_2O_5: K_2O ha^{-1}) the number of rows per cob

was found to be 14.6 and the significantly least number of rows per cob (12.6) was recorded in the treatment T_1-120:60:60 kg N: P_2O_5: K_2O ha^{-1}. These results corroborated with the findings of Umesh et. al. (2014). The numbers of grains per row were 31.67 in T_8 and the lowest number of grains per row (27.48) was recorded in T_1 (Table 3). This finding was similar to the results reported by Joshi et al. (2016).

Table 1. Growth parameters at harvest as influenced by nutrient management practices in hybrid maize crop

Treatments	Plant height (cm)	Green leaves per plant (no.)	Leaf Area Index	Dry matter (g m^{-2})
T_1-120:60:60 kg N:P_2O_5:K_2O kg ha^{-1}	194.67	10.53	1.62	1234.25
T_2-120:60:60 kg N:P_2O_5:K_2O kg ha^{-1} + $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	196.27	10.87	1.77	1252.54
T_3-150:75:75 kg N:P_2O_5:K_2O kg ha^{-1}	198.53	11.00	1.85	1277.93
T_4-150:75:75 kg N:P_2O_5:K_2O kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}	201.07	11.27	1.87	1306.53
T_5-180:90:90 kg N:P_2O_5:K_2O kg ha^{-1}	204.93	11.33	1.94	1327.98
T_6-180:90:90 kg N:P_2O_5:K_2O kg ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1}	206.27	11.53	2.02	1348.69
T_7-STBFR(150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + ZnSO4 @ 25 kg ha^{-1} + Borax @10 kg ha^{-1}	212.87	11.73	2.26	1423.76
T_8-STCR (216: 15: 21 kg N: P_2O_5: K_2O kg ha^{-1})	209.60	11.67	2.17	1381.81
SEm (±)	1.27	0.08	0.028	2.01
CD (P=0.05)	3.88	0.23	0.061	6.16

Table 2. Number of days to 50% tasseling and 50% silking as influenced by nutrient management practices in hybrid maize crop

Treatments	50 % tasseling	50% silking
T_1-120:60:60 kg N:P_2O_5:K_2O ha^{-1}	53.33	54.33
T_2-120:60:60 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	53.33	54.67
T_3-150:75:75 kg N:P_2O_5:K_2O ha^{-1}	53.67	55.00
T_4-150:75:75 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1}	54.00	55.67
T_5-180:90:90 kg N:P_2O_5:K_2O ha^{-1}	54.33	55.67
T_6-180:90:90 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$@25 kg ha^{-1} + Borax@10 kg ha^{-1}	54.67	56.33
T_7-STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1}	56.33	58.00
T_8-STCR (216: 15: 21 kg N: P_2O_5: K_2O ha^{-1})	55.00	57.33
SEm (±)	0.52	0.54
CD (P=0.05)	1.61	1.66

Table 3. Effect of nutrient management practices on yield attributing characters in hybrid maize crop

Treatments	Cob length (cm)	Cob girth (cm)	No. of rows per cob	No. of grains Per row	1000 grain weight (g)
T_1-120:60:60 kg N:P_2O_5:K_2O ha^{-1}	15.48	14.09	12.6	27.48	269.75
T_2-120:60:60 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	15.93	14.19	13.0	28.73	272.31
T_3-150:75:75 kg N:P_2O_5:K_2O ha^{-1}	16.38	14.31	13.2	28.26	274.65
T_4-150:75:75 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$@25 kg ha^{-1}+Borax@10 kg ha^{-1}	16.57	14.43	13.6	28.61	276.39
T_5-180:90:90 kg N:P_2O_5:K_2O ha^{-1}	16.83	14.66	13.8	29.52	277.82
T_6-180:90:90 kg N:P_2O_5:K_2O ha^{-1} +$ZnSO_4$@25 kg ha^{-1}+Borax@10 kg ha^{-1}	17.18	14.86	14.2	30.76	279.46
T_7-STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1})+$ZnSO_4$@25 kg ha^{-1} +Borax @10 kg ha^{-1}	18.06	15.94	15.2	32.13	282.74
T_8-STCR (216: 15: 21 kg N: P_2O_5: K_2O ha^{-1})	17.55	15.67	14.6	31.67	281.59
SEm (±)	0.09	0.14	0.19	0.29	2.42
CD(P=0.05)	0.29	0.42	0.51	0.92	7.52

Nutrient management practices influenced the grain yield significantly. Significantly maximum grain yield of 8256 kg ha^{-1} was obtained with the application of STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} inT_7 followed by STCR (216: 15: 21 kg N: P_2O_5: K_2O ha^{-1}) fertilizer application in T_8, where the grain yield recorded was 8184 kg ha^{-1} which were at par with each other. This yield variation was due to differences in nutrients applied and variation among the yield attributes recorded in the respective treatments in Table 4. It corroborates the findings of Singh and Patiram (2012). Enhancement in growth attributes led to photosynthesis partitioning and better source-sink relationship, which enhanced yield attributes (Kumar et al., 2015). Stover yield recorded in different treatments also followed similar trend as grain yield. The variation in stover yield due to different nutrient combinations was attributed to consequent variations in plant height, number of green leaves and total dry matter accumulation capacity of the treatments. Harvest index (HI) increased progressively with nutrients management practices significantly. Among the nutrient management practices, application of STCR (216: 15: 21 kg N: P_2O_5: K_2 O ha^{-1}) in T_8 recorded the significantly highest HI of 44.40 which was at par with T_7-STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} (44.36).

The treatment T_7-STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} gave the maximum gross return of Rs. 141678 ha^{-1} (Table 5), whereas RDF recorded significantly less gross return of Rs. 97597 ha^{-1}. The increase of gross return by T_7 over T_8 and T_1 were 1.10 and 1.45 % respectively. The increase in net return due to STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @ 10 kg ha^{-1} in T_7 over T_8- STCR (216: 15: 21 kg N: P_2O_5: K_2O ha^{-1}) and T_1-120:60:60 kg N: P_2O_5 :K_2O ha^{-1} were 1.11 and 1.61 % respectively. Highest benefit: cost ratio of 3.33 was recorded in T_7 followed by T_8 and T_1. This was in conformity with the findings of Chander et al. (2013), where soil test-based nutrient balancing was done through the application of nitrogen, phosphorus, and potassium in addition to sulphur, boron, and zinc increased crop productivity and hence proved favorable to scale-up balanced nutrition.

Table 4. Effect of nutrient management practices on yield of hybrid maize crop

Treatments	Grain yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest Index (%)
T_1-120:60:60 kg N:P_2O_5:K_2O ha^{-1}	6737	9644	41.12
T_2-120:60:60 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	6867	9692	41.47
T_3-150:75:75 kg N:P_2O_5:K_2O ha^{-1}	7293	9812	42.62
T_4-150:75:75 kg N:P_2O_5:K_2O ha^{-1} +$ZnSO_4$@25 kg ha^{-1}+Borax@10 kg ha^{-1}	7503	9950	42.98
T_5-180:90:90 kg N:P_2O_5:K_2O ha^{-1}	7654	10015	43.31
T_6-180:90:90 kg N:P_2O_5:K_2O ha^{-1} +$ZnSO_4$@25 kg ha^{-1}+Borax@10 kg ha^{-1}	7892	10136	43.77
T_7-STBFR(150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$@25 kg ha^{-1} + Borax @10 kg ha^{-1}	8256	10353	44.36
T_8-STCR (216: 15: 21 kg N: P_2O_5: K_2O ha^{-1})	8184	10247	44.40
SEm (±)	79.04	99.56	0.61
CD (P=0.05)	247.66	389.30	1.9

Table 5. Economics of production as influenced by different approaches of fertilizer management practice in hybrid maize crop

Treatments	Cost of cultivation (Rs. ha^{-1})	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	B:C Ratio
T_1- 120:60:60 kg N:P_2O_5:K_2O ha^{-1}	36000	97597	61597	2.71
T_2- 120:60:60kg N:P_2O_5:K_2O ha^{-1}+ $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	39500	108931	69431	2.76
T_3- 150:75:75 kg N:P_2O_5:K_2O ha^{-1}	37500	109207	71707	2.91
T_4- 150:75:75 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1}	40500	121207	80707	2.99
T_5-180:90:90 kg N:P_2O_5:K_2O ha^{-1}	40500	124391	83891	3.07
T_6-180:90:90 kg N:P_2O_5:K_2O ha^{-1} + $ZnSO_4$ @ 25 kg ha^{-1}+ Borax @ 10 kg ha^{-1}	43500	138459	94959	3.18
T_7-STBFR(150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25kg ha^{-1} + Borax @ 10 kg ha^{-1}	42500	141678	99178	3.33
T_8-STCR (216: 15: 21 kg N: P_2O_5:K_2O ha^{-1})	40000	129046	89046	3.23

CONCLUSION

Thus it could be concluded that application of STBFR (150: 39: 45 kg N: P_2O_5: K_2O kg ha^{-1}) + $ZnSO_4$ @ 25 kg ha^{-1} + Borax @10 kg ha^{-1} produced the maximum grain yield of 8256 kg ha^{-1} and stover yield of 10353 kg ha^{-1} in hybrid maize during *kharif* season. The growth parameters and yield attributes followed the same pattern as that of grain yield. It recorded highest net return and benefit: cost ratio of Rs. 99178 per ha and 3.33 respectively. For efficient hybrid maize production in acid sandy loam soil low in organic carbon under Bhubaneswar agro-climatic condition higher fertilizer dose than the recommended according to the soil test based recommendation need to be applied i.e. Soil Test Based Fertilizer Recommendation (STBFR) and addition of Zn and B are essential for maintaining the macro and micronutrient and to improve and sustain productivity and quality of hybrid maize grown during *kharif* season.

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Effect of nitrogen on yield and yield attributes of soybean in Kunar, Afghanistan

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ABSTRACT

A field experiment was conducted at Research farm of Agriculture Faculty of Sayed Jamaluddin Afghani University in Kunar during 2020 to evaluate the effect of nitrogen rates and time of applications on yield and yield attributes of soybean. The experiment was laid out in Randomized complete block design (RCBD) and replicated thrice consisted of seven treatments Viz; T_1: Control, T_2: 12.5 kg N ha^{-1} as basal, T_3:25 kg N ha^{-1} as basal, T_4: 50 kg N ha^{-1} as basal, T_5: 6.25 kg N ha^{-1} as basal + 6.26 kg N ha^{-1} at flowering Stage, T_6:12.5 kg N ha^{-1} at basal + 12.5 kg N ha^{-1} at flowering stage and T_7: 25 kg N ha^{-1} at basal + 25 kg N ha^{-1} at flowering stage. The highest reproductive attributes like pod length, pods per plant, seeds per pod, seed index, grain and straw yield were found in T_7 which was statistically similar to T_6 and the lowest values of all the parameters were obtained from control treatment. The overall finding of this study indicated that 25 kg N ha^{-1} at basal + 25 kg N ha^{-1} at flowering stage can be applied to achieve better yield attributes and yield of soybean.

Key words: Nitrogen, soybean, rates and time of applications, yield and yield attributes

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the most important oilseed and grain legume corp rich in protein and is cultivated in many parts of the world. In the international trade markets, soybean is ranked number one in the world among the major oil crops. Worldwide the cultivation area for soybean is 122.30 million hectare with average productivity of 2.70 tonnes ha^{-1} and total production of 330.41 million metric tonnes production (FAO, 2019). In Afghanistan, it is also one of the most important crops for human being. It is used as soy naan, soy dishes, including soybean Korma (soup), soymilk and full-fat soy flour and soybeans for pregnant and lactating women. The production of soybean was record 4,000 metric tonnes with average productivity of 0.6 to 1.2 tonnes ha^{-1} (NEI, 2015; NEI, 2011). Yield of soybean is very low in Afghanistan due to number of reasons, viz. lack of high yielding varieties, fertilizer management and delayed sowing (Gathiye et al., 2020). Without N fertilization formers cannot achieve high yield. Nitrogen is essential nutrients as they are involved in many biochemical processes and their deficiency not only affects crop productivity but also the quality of produce for human and

animal consumption (Salvagiotti et al. 2009; Hari Krishnan and Jez, 2018). Nitrogen fertilizer has had positive effects on soybean growth and yield (Brevedan et al., 1987; Touchton and Ricker, 1986). Soybean yield can be increased by adding nitrogen fertilizers in critical growth stages of soybean. Split application of nitrogen supplies nitrogen according to the demand of a growing crop and can improve nitrogen use efficiency. Splitting N applications may decrease the loss of N applied at planting due to denitrification. Split application reduces the risk of losses as leaching. Split application of nitrogen can increase grain yield and grain protein content (Laharia et al., 2013). In Afghanistan, as per the NEI recommendation 50 kg urea ha^{-1} was applied in soybean at sowing and at flower initiation stage. Therefore, the study was conducted to find out the suitable combination of nitrogen rate and time of application to obtain better yield attributes and yield of soybean.

MATERIALS AND METHODS

A field experiment was conducted at Research Farm of Agriculture Faculty, Sayed Jamaluddin Afghani University (SJAU), Kunar, Afghanistan (34º 83' N latitude; 71º 12' E longitude) from June to Oct, 2020. Soybean high yielding variety (Hwang keum) was sown in the experiment. The experiment was laid out in Randomized Block Design with three replications. There were seven treatments Viz; T_1: Control, T_2: 12.5 kg N ha^{-1} as basal, T_3:25 kg N ha^{-1} as basal, T_4: 50 kg N ha^{-1} as basal, T_5: 6.25 kg N ha^{-1} as basal + 6.26 kg N ha^{-1} at flowering Stage, T_6:12.5 kg N ha^{-1} at basal + 12.5 kg N ha^{-1} at flowering stage and T_7: 25 kg N ha^{-1} at basal + 25 kg N ha^{-1} at flowering stage. The size of the individual plot was 3.5 m x 5m and total numbers of plots were 21. The different doses of nitrogen were applied as per treatment through urea. The dose and time of applications were kept as per the treatments. All the recommended cultural practices and inputs including fertilizers were applied uniformly to all the plots. Finally, plants were harvested and data on yield and yield attributes parameters were collected from crop samples. Grain yield and straw yield altogether were considered as biological yield. Harvest index denotes the ratio of grain yield to biological yield multiplied by 100. Analysis of variance was done with the help of computer package program according to Gomez and Gomez (1984) and the mean differences among different treatments were adjudged by C.D test.

RESULTS AND DISCUSSION

Effects of treatment on reproductive attributes

Significant variation was measured in case of pod length of soybean due to application of different nitrogen rates and application times (Table 1).

Table 1. Yield and yield attributes of soybean as influenced by different nitrogen rates and time of applications

Treatment	Pod length (cm)	Pods per plant (no.)	Seeds per pod (no.)	Seed index (g)	Seed yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest index (%)
T_1	4.09	88.00	2.00	12.00	1.38	4.31	32.01
T_2	4.62	115.67	2.22	12.00	1.53	4.83	31.75
T_3	4.77	158.11	2.44	12.55	162	4.88	33.16
T_4	4.61	168.78	2.78	12.78	1.68	5.42	30.95
T_5	4.68	136.67	2.33	12.63	1.85	6.09	30.43
T_6	4.84	200.22	2.78	13.30	2.04	6.33	32.25
T_7	5.16	247.22	2.89	13.26	2.15	6.94	30.99
C.D. 5 %	0.64	88.00	0.40	NS	0.15	0.38	NS

The longest pod was observed in T_7 which was statistically similar with the rest of the treatments except T_1. However, the shortest pod was recorded from T_1. Nutrient elements ensure maximum accumulation of photo-synthates to the pod, thus increase the length of pod. These findings are in accordance with the results of Khaim et al. (2013).

Analysis of variance showed that number of pods per plant of soybean showed statistically significant variation for the application of different nitrogen application rates and time of applications (Table 1). The highest number of pods was recorded in T_7 which was found statistically similar with T_6, while the minimum number of pods per plant from T_1. This finding was supported by Begum et al. (2015) who observed that increasing nitrogen rate increased the number of pods per plant.

Number of seeds was exposed significantly with different nitrogen rates and application times (Table 1). The maximum number of seeds pod^{-1} was recorded from T_7 treatment, which was statistically similar to T_6 and T_4 treatments, whereas the minimum was recorded from T_1, which was at par with T_2, T_3 and T_5. This finding is consistence with the finding of Patil and Udmale (2016).

Seed index (g)

Seed index of soybean did not varied significantly with different rates and time of nitrogen application (Table 1). The maximum seed index of soybean was weighted from T_6 treatment whereas the minimum was recorded from T_1.

Grain yield

Yield of soybean varied significantly with different rates and time of nitrogen applications (Fig. 1). The highest yield of soybean was obtained from T_7, which was statistically similar with T_6 whereas the lowest was obtained from T_1. The results showed that application of nitrogen at later growth stages resulted in higher grain yield as compare to nitrogen application at sowing alone. This might be due to proper applying of nitrogen in later stages which help in increasing crop yield. Caliskan, 2008 reported that 40 kg N ha^{-1} application of starter and top dressed nitrogen can be beneficial to improved yield of soybean. Khalili et al., 2016 also reported that application of N rates and time of applications increased grain and stover yield of maize over control

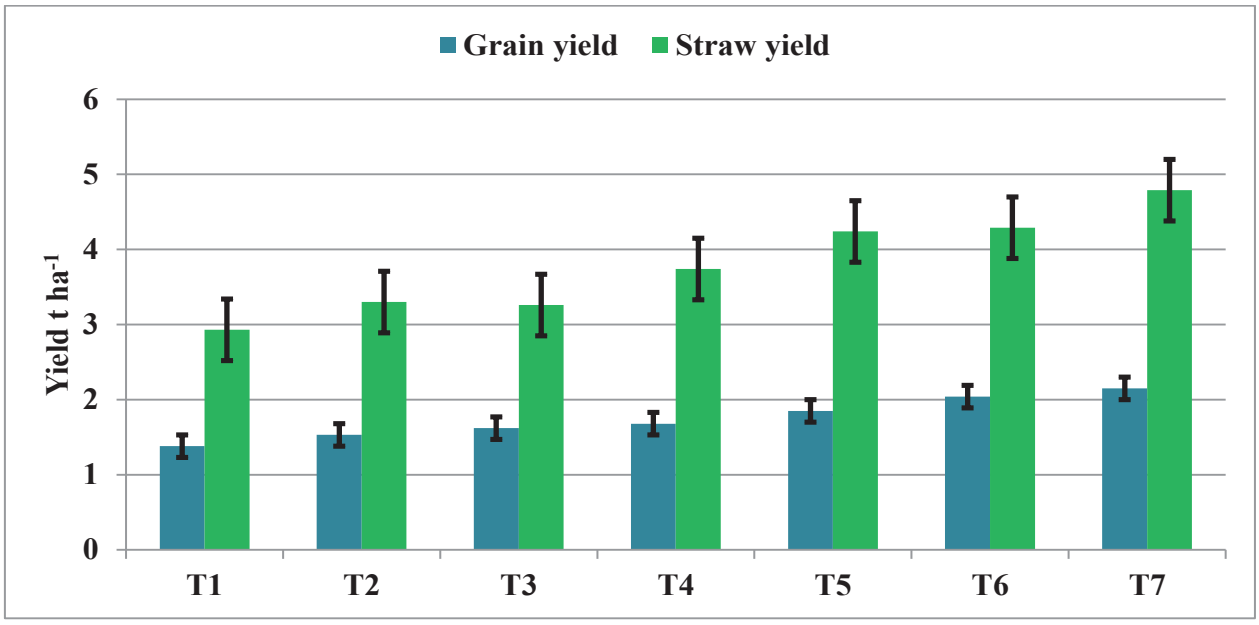

Fig. 1. Grain and stover yield of soybean as influenced by different nitrogen rates and time of applications

Straw yield

This research work exhibited distinct variation in straw yield of soybean with different rates and time of nitrogen application (Fig. 1). The maximum straw yield of soybean was produced from T_7 followed by T_6 and T_5. However, the minimum was produced from T_1. Khaim et al. (2013) observed that application of organic and inorganic fertilizers increased the stover yield of soybean.

Biological yield

It is evident from (Table 1) that biological yield was significantly different with rates and time of nitrogen application. The highest biological yield was found in T_7 followed by T_6 and T_5, while the lowest was found in T_1.

Harvest index (%)

Harvest index of soybean was not varied significantly due to various treatments of nitrogen rates and time of applications (Table 1). However, numerically the highest harvest index was obtained from T_3 and the lowest harvest index of soybean was recorded from T_5. The results are in contrary with the results of Khaim et al. (2013) who reported that harvest index was influenced by the application of organic and inorganic fertilizer.

CONCLUSION

Nitrogen rates and time of applications significantly affected soybean yield attributes and yield. Superior yield attributes and yield of soybean can be obtained with the application 25 kg N ha^{-1} at basal + 25 kg N ha^{-1} at flowering stage.

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Genetic variability in blackgram [*Vigna mungo* (L.) Hepper] under acid soils of Manipur

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ABSTRACT

The experimental material comprising twenty four genotypes of black gram were grown during kharif 2018. The analysis of variance revealed that all the characters are significant except pod length and number of seeds per pod at 5% level. Moderate PCV and GCV value were found for days to fifty per cent flowering and number of cluster per plants, shows the influence of environmental factors. Both genotypic and phenotypic correlations were calculated and the results revealed that, genotypic correlation value is more than phenotypic correlation. Yield per plot is showing positive correlation with 100 seed weight. The path analysis shows that Plant height, number of pods per plant and number of seed per pod showed influential effect on seed yield per plant. The genotypes in the cluster V had maximum cluster per plant, whereas maximum number of seed per pod and pod length were revealed by the genotypes in the cluster VI. The characters viz., number of pods per plant (23.91%), 100 seed weight (18.84%), yield per plot (12.68%), days to 50% flowering (11.96%) and pod length (11.96%) showing major contributions. Therefore, the choice of suitable diverse parents selected on the basis of genetic divergence analysis became more rewarding than the choice made on the basis of geographic diversity as reported in the earlier findings.

Key words: Blackgram, GCV, genetic variability, heritability, PCV

INTRODUCTION

Blackgram [*Vigna mungo* (L.)] Hepper is one of the important kharif pulses grown in the hill and upland area of Manipur and other North Eastern states. Culturally, blackgram holds lot of importance in Manipur society. Blackgram is well suited for cultivation in the hilly terrain of NEH region. It prevents the soil losses due to the soil erosion in the hilly area. Genetic diversity is a vital aspect in any crop improvement program for delivering high yielding variety. Suitable varieties are not available in Manipur for cultivation. So, multivariate analysis such as D^2 cluster has been proved to be useful in selecting advanced genotypes for hybridization. Mahalanobis (1949) D^2 analysis has been efficiently used in measuring the diversity in several crops. An understanding of nature and degree of variability among the existing blackgram germplasm is a precondition for its improvement. Genetic divergence analysis is a useful tool in quantifying the degree of divergence between biological population of geographical level and to access in assessing relative contribution of different components to the total divergence both at intra and inter cluster levels (Jatasra and Paroda, 1978). Accurate information on the nature and degree of genetic divergence helps the plant breeder in choosing the varied parents for purposeful hybridization (Arunachalam, 1981; Samsuddin, 1985). The present investigation has been undertaken with twenty four black gram genotypes

from all the Indian states to understand the different variability factors, association between the traits, nature and magnitude of genetic divergence and the characters contributing genetic diversity by correlation coefficient, path and D2 analysis.

MATERIALS AND METHODS

The experimental material comprising twenty four genotypes of blackgram were grown during kharif 2018 in a randomized block design with three replications at Andro Research Farm, CAU, Imphal. Each genotype was sown in five rows of 3 meter length spaced at 30 cm; and 10 cm between the plants. All the recommended package of practices was adopted to raise a good crop. The data on nine yield contributing parameters viz., days to fifty per cent flowering, days to maturity, plant height, number of cluster per plant, number of pods per plant, number of seeds per plant, pod length, 100 seed weight and yield (gram) per plot were observed. Analysis of variance for the observations recorded on different characters was carried out as per the standard procedure of Fisher (1938). Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953). Heritability in broad sense was worked out as per the procedures of Falconer (1981). The analysis of genetic divergence was carried out by using Mahalanobis D2 statistic as per Mahalanobis (1936) method and genotypes were grouped into different clusters following Tochers method as described by Rao (1952).

RESULTS AND DISCUSSION

In the present study, the analysis of variance revealed that all the characters are significant except pod length and number of seeds per pod at 5% level. In the present study, the phenotypic coefficient of variation (PCV) was found higher than that of genotypic coefficient of variation (GCV) for all the traits (Table 1), suggesting the role of environmental factors on various characters, also suggested by Khajudparn et al., (2012). Moderate PCV and GCV value were found for days to fifty percent flowering and number of cluster per plants, shows the influence of environmental factors. All other characters were showing the lowest GCV and PCV values indicate that the simple phenotypic selections are not effective for these characters. Moderate heritability were shown by number of pods per plant, 100 seed weight, pod length, yield plot and days to fifty per cent flowering shows that the characters were governed by the non additive gene action. Similar results were obtained by Chauhan et al. (2020) and Punia et al. (2014).

Table 1. Estimates of mean performance, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (broad sense) for yield contributing characters in blackgram

Character	Mean	S.E	C.D (5%)	CV (%)	PV	GV	EV	h2 (%)
DFF	38	0.67	1.89	3.00	2.55	1.22	1.33	47.83
DM	77	2.38	6.76	5.34	20.70	3.72	16.97	17.99
Pht	6.53	0.13	0.36	3.35	0.05	0.00	0.05	7.47
NCP	27.69	2.11	5.99	13.19	16.27	2.93	13.34	18.01
NPP	4.76	0.31	0.89	11.39	0.81	0.51	0.29	63.65
PL	14.03	0.98	2.77	12.05	5.86	3.00	2.86	51.23
NSP	4.75	0.13	0.38	4.91	0.07	0.02	0.05	24.93
100 sw	4.70	0.15	0.42	5.39	0.15	0.09	0.06	57.31
Yd/plot(g)	500	46.20	131.32	16.00	12516.05	6113.24	6402.81	48.84

*DFF-Days to 50% flowering, DM-Days to maturity, NCP- Number of clusters per plant, NPP- Number of pods per plant, PL- Pod length, NSP-Number of seeds per pod, Pht- plant height, 100 sw- 100 seed weight(g) Yd/plot(g)- yield per plot, PV-Phenotypic coefficient of variation, GV- Genotypic coefficient of variation, EV- environment coefficient of variation and h2(%)- heritability

The information on correlation of the quantitative characters with related characters is the prime criteria to form an effective selection strategy expected for its improvement. Therefore, to determine the relative importance of the component traits and to initiate an effective selection programme, correlation analysis was done. Both genotypic and phenotypic correlations were calculated and the results revealed that, genotypic correlation value is more than phenotypic correlation (Table 2). Days to fifty per cent flowering showed significant and positive correlation with days to maturity, plant height, pod length and 100 seed weight. Similarly Days to maturity shows significant and positive trend with plant height, and 100 seed weight. Number of cluster per plant shows positive correlation with number of pods per plant. The positive and significant correlation was shown by number of seeds per pod with pod length. Yield per plot is showing positive correlation with 100 seed weight. Similar results were obtained by Miah et al. (2016), Sohel et al. (2016), Shalini and Lal (2019) and Shanthi et al. (2019).

Table 2. Genotypic and phenotypic correlation coefficients among the yield contributing characters in blackgram

Character	Correlations	DM	Pht	NCP	NPP	NSP	PL	100 seed weight	Yield/ plot (g)
DFF	Geno	**0.76****	**1.13****	0.09	0.07	0.17	**0.92****	**0.62****	0.14
	Pheno	0.19	0.19	0.12	0.19	0.04	0.08	0.31	-0.07
DM	Geno		**0.87****	0.04	0.06	-0.15	0.14	**0.64****	0.19
	Pheno		0.30	0.02	-0.04	0.08	0.21	0.13	0.21
Pht	Geno			0.17	0.014	0.16	**0.43****	**1.02****	0.09
	Pheno			0.16	0.11	0.14	0.14	0.38	0.31
NCP	Geno				**0.87****	-0.56	-0.72	0.08	-0.02
	Pheno				**0.84****	-0.10	-0.08	0.03	0.08
NPP	Geno					-0.74	**-0.82****	-0.10	-0.03
	Pheno					-0.19	-0.15	-0.04	0.08
NSP	Geno						**1.08****	0.04	0.14
	Pheno						**0.85****	0.12	0.17
PL	Geno							0.05	0.07
	Pheno							0.23	0.22
100 swt	Geno								**0.43***
	Pheno								0.35

*- Significant at 5% level; **-Significant at 1% level

**DFF-Days to 50% flowering, DM-Days to maturity, NCP- Number of clusters per plant, NPP- Number of pods per plant, PL- Pod length, NSP-Number of seeds per pod, Pht- plant height, 100 sw- 100 seed weight(g) Yd/plot(g)- yield per plot

Table 3 shows that Plant height, number of pods per plant and number of seed per pod showed positive direct effect on seed yield per plant. The character days to 50% flowering had positive indirect effect to days to maturity, plant height, number of pods per plant, number of seeds per pod and 100 seed weight. Days to maturity shows positive indirect effect through days to maturity, plant height, number of pods per plant and 100 seed weight on seed yield per plant reconstitute the scentence. The character of plant height influence high negative indirect effect via days to 50 per cent flowering. Number of cluster per plant shows high positive effect through number of pods per plant. Number of pods per plant shows high positive direct on seed yield per plant and high negative

effect through number of cluster per plant and number of seed per pod. This result is corroborated with Punia et al. (2014) and Shivade et al. (2011) in blackgram. Number of seeds per pod negatively influences the yield per plot indirectly through days to 50% flowering, days to maturity, number pods per plant and pod length. Pod length shows highest positive indirect effect through number of seed per pod and negatively influenced through days to 50% flowering, number of pods per plant and pod length. The character 100 seed weight has an indirect positive influence on yield per plot through plant height. Umadevi and Meenakshi (2005) reported positive direct effect of number of clusters per plant on seed yield per plot. The positive direct effect of number of pods per plant was nullified by negative indirect effects through number of clusters per plant, days to first flowering, number of seed per pod and 100 seed weight which resulted in the insignificant negative association with seed yield per plant. The strong positive association of number of seed per pod was observed due to its positive direct effects on seed yield per plant and positive indirect effect through plant height, number of cluster per plant and 100 seed weight. Kingshlin and Vanniarajan (2000), Srividhya. et al. (2005), Sohel et al. (2016), Prasanna and Gabrial (2018) and Singh et al. (2019) reported positive direct effect for these characters in blackgram.

Table 3. Direct and indirect genotypic effect of nine quantitative characters on seed yield in black gram

Character	DFF	DM	Pht	NCP	NPP	NSP	PL	100 sw	Yd/plot
DFF	-0.6452	0.0893	0.9186	-0.0776	0.0835	0.1724	-0.4545	0.0528	0.1395
DM	-0.4881	0.1181	0.7045	-0.0363	0.0634	-0.1518	-0.0714	0.0544	0.1927
Pht	-0.7285	0.1023	0.8135	-0.1531	0.0155	0.1639	-0.2118	0.087	0.0888
NCP	-0.0564	0.0048	0.1404	-0.8871	0.9804	-0.5606	0.3529	0.0065	-0.0192
NPP	-0.0479	0.0067	0.0112	-0.7733	1.1248	-0.7451	0.4053	-0.0085	-0.0268
NSP	-0.1111	-0.0179	0.1332	0.4968	-0.8371	1.0011	-0.5314	0.0032	0.1368
PL	-0.5938	0.0171	0.3489	0.6340	-0.9232	1.0774	-0.4938	0.0039	0.0704
100 swt	-0.3999	0.0754	0.8314	-0.0673	-0.1126	0.038	-0.0225	0.0851	0.4277

*DFF-Days to 50% flowering, DM-Days to maturity, NCP- Number of clusters per plant, NPP- Number of pods per plant, PL- Pod length, NSP-Number of seeds per pod, Pht- plant height, 100 sw- 100 seed weight(g) Yd/plot(g)- yield per plot.

The genetic diversity analysis was carried out in such advanced breeding line collection helped to exploit the wealth of information in crop improvement programmes and for managing the germplasm without any loss in its potential. The genetic divergence was studied in the germplasm of 24 genotypes of blackgram by Mahalanobis D2 analysis. By using of clustering techniques, the 24 genotypes were grouped into six distinct clusters (Table 4).

Table 4. Cluster composition of twenty eight blackgram genotypes (Tocher's method)

Cluster no	No of genotypes	Genotypes name
I	10	IPU 12-5, VBG12-110, RU03-22-4, Uttara, PU1501, PU1541, IPU17-1, KU17-04, AKU1608, MU52
II	6	OBG41,DKU90, KPU514-75, DBGV16, OBG103, TBG129
III	3	LBG904, VBG17-026, GJU1509
IV	3	KPU 52-87, SBC 50, KUG818
V	1	MBG1070
VI	1	Pant U 31

Cluster I had got the maximum numbers of genotypes (10 genotypes). The second group having 6 genotypes, third and fourth clusters consisted of three genotypes each and the remaining 2 clusters were mono genotypic. The grouping pattern of genotypes indicated the presence of substantial diversity in blackgram germplasm. The genotypes included in a cluster were from diverse geographical origin indicating that the geographic diversity need not be necessarily related to genetic diversity. Therefore these results are in conformity to those reported by Verma and Kalna (1997), Manivannan et al. (1998) and Dikshit and Swain (2002).

The intra and inter-cluster average distances among six clusters during *kharif*, 2018 is presented in Table 5. The maximum intra-cluster distance (D_2) was registered for cluster IV (14.50) and cluster II (13.61). Critical perusal of the data presented in Table 5 shown that maximum inter-cluster distance (D2) was found between cluster Vand VI (97.92) followed by between cluster III and VI (53.7). Highly divergent groups shows the maximum inter cluster distance. The inter cluster distance must be taken into consideration while selecting the parents for a hybridization program. It is assumed that maximum amount of heterosis manifested in cross combination involving the genotypes belonging to most divergent clusters. These results are corroborated with the findings of Umadevi and Meenakshi (2007), Chauhan et al. (2008) and Jeberson et al. (2019).

Table 5. Average of intra (in bold) and inter cluster D_2 distances

Cluster	I	II	III	IV	V	VI
I	11.45	19.06	20.67	31.10	24.49	44.97
II		13.61	28.54	21.76	37.12	34.91
III			12.19	42.10	44.91	53.71
IV				14.50	46.92	46.10
V					0.00	97.92

The mean performance of all the characters in different cluster is presented in Table 6. The results clearly underlines that different clusters showed wide variation from one another in respect of cluster means. This shows that genotypes having distinctly different mean performance for different traits were separated in to different clusters. Mean values of different clusters revealed wide range of differences between clusters. The genotypes in the cluster V had maximum cluster per plant, whereas maximum number of seed per pod and pod length was revealed by the genotypes in the cluster VI. The shortest plant height was recorded for cluster IV and VI. Thus, these genotypes have a potential to be used as parental stock in hybridization program. Thus upon hybridization between these lines, we can create genetic variance for selection. The factors responsible for differentiation of intra- and inter-cluster levels were influenced by environments as indicated by cluster means of various characters (Patil et al., 2003). On the basis of this grouping it may be concluded that an effective hybridization program can be initiated including the genotypes of diverse group to produce better per segregates which can be used for the development of high yielding blackgram varieties in future.

Table 6. Cluster means for nine (9) quantitative characters studied in Blackgram

CLUSTER	DFF	DM	Pht	NCP	NPP	NSP	PL	100 sw	Yd/plot
I	38	77	28.0	5.2	14.9	4.7	6.5	4.9	544
II	38	76	26.6	4.3	12.3	4.8	6.5	4.5	443
III	41	80	31.1	4.6	13.3	4.8	6.6	5.0	499
IV	38	76	25.5	4.3	14.9	4.7	6.6	4.3	412
V	38	79	29.8	6.9	18.5	4.7	6.5	4.5	451
VI	37	74	25.5	3.3	10.5	5.0	6.7	4.5	722

Table 7. Relative contribution of 9 traits towards divergence

Character towards divergence	Number of First Ranks	Percentage of contribution
DFF	33	11.96
DM	15	5.43
NCP	8	2.90
NPP	66	23.91
PL	33	11.96
NSP	25	9.06
Pht	9	3.26
100 sw	52	18.84
Yd/plot(g)	35	12.68

*DFF-Days to 50% flowering, DM-Days to maturity, NCP- Number of clusters per plant, NPP- Number of pods per plant, PL- Pod length, NSP-Number of seeds per pod, Pht- plant height, 100 sw- 100 seed weight(g) Yd/plot(g)- yield per plot

Table 7 shows the relative contribution of traits towards the genetic diversity, out of which number of pods per plant (23.91%), 100 seed weight (18.84%), yield per plot (12.68%), days to 50% flowering (11.96%) and pod length (11.96%) showing major contributions. Characters which are responsible for more divergence will be given more priority for the purpose of further selection and the choice of parents for hybridization (Srividya et al., 2018).

CONCLUSION

It can be concluded from the present investigation that the characters, number of cluster per plants, number of pods per plant and 100 seed weight are important traits for the improvement of blackgram. According to the D2 analysis the grouping of twenty four genotypes into six distinct clusters indicates that considerable diversity existed in the genotypes studied which are in agreement with earlier reports indicating substantial diversity in blackgram material. The above findings clearly show that different clusters exhibited extensive variation from one another in respect of cluster means. This indicated that genotypes having distinctly different mean performance for various characters were separated in to different clusters. The instances of grouping of genotypes of different origin or geographic region in same cluster were frequently observed. This suggested that there is no parallelism between genetic and geographic diversity. Therefore, the choice of suitable diverse parents selected on the basis of genetic divergence analysis would be more rewarding than the choice made on the basis of geographic diversity as reported in the earlier findings.

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Effect of phosphorus levels on soil carbon fractions in acid soil

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ABSTRACT

A pot experiment carried out to understand the effect of phosphorous (P) application on different soil carbon fraction in acidic soil at the research farm of College of PG Studies in Agricultural Sciences (CPGS-AS), CAU (I) revealed that the microbial carbon fraction (MBC) and particulate organic matter (POM) were increased with the application of phosphorous. About 16.67% soil organic carbon (SOC), 19.09% oxidizable organic carbon fraction 1 (OOC1), 23.46% oxidizable organic carbon fraction 2 (OOC2) and 16.67% oxidizable organic carbon fraction 3 (OOC3) were decreased with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0(NPK @ 80:0:40 kg ha^{-1}). Dissolved organic carbon (DOC) was decreasing 16.73% with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0(NPK @ 80:0:40 kg ha^{-1}). However, 5% hot water extractable carbohydrates (HWC) and 2.4% water extractable carbohydrates (WC) were increased with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0. The SOC, OOC2, OOC3 at 30 DAS and OOC3 at crop maturity were decreasing. The SOC and OOC2 at crop maturity were finally gained its content at crop maturity. The POM, DOC, MBC and WC were lower at crop maturity than those at 30 DAS.

Key words: Available phosphorous, land use, soil organic carbon, topography, Umiam

INTRODUCTION

Considerable amount of nutrients loses from the soil every year in North Eastern Hill (NEH) region of India due to hilly terrain (77% hilly), very high rainfall (>2000 mm per annum) and shifting cultivation practice which also contribute for the development of soil acidity. Soil organic carbon (SOC) is an index of soil fertility, capacity of a soil to supply nitrogen (N), phosphorous (P), sulfur (S) and trace metals to plants (Pan et al., 2009), however, the SOC decomposition might inhibit or decrease with nutrient limitation and C:N:P ratio (Zhu et al. 2018). Microbial activities would be increased with nutrients sufficiently meet microbial C:N:P ratio requirements and SOC mineralization stimulated (Wei et al. 2017, 2019). Soil microorganisms determine the magnitude and direction of SOC mineralization (Yuan et al., 2016; Li et al., 2018). The change in SOC mineralization rate might enhance the utilization of labile resources by microorganisms (Fontaine et al., 2003). The soil management has a significant impact on soil carbon (C) fractions and N turn over and also has an effect on P dynamics. Labile soil organic carbon fractions like dissolved organic carbon (DOC), microbial biomass C (MBC), and particulate organic carbon (POC) are the fine indicators of soil quality which influence soil function and are sensitive to change in soil management practices (Xu et al., 2011; Nayak et al., 2012). The labile fraction consists of material in transition between fresh plant residues and stabilized organic matter. On the other hand, stabilized fraction of SOM is composed of organic

materials that are highly resistant to microbial decomposition (Haynes, 2005). Labile fractions are the potential indicator of soil quality changes due to management practices (e.g. tillage, fertilizer and manure applications) (Marriott and Wander, 2006; Yoo et al., 2006; Sharifi et al., 2008). Measurement of labile fractions is required for a better assessment of the effects of management on soil properties (Iovieno et al., 2009) and improved understanding of labile organic matter fractions will provide valuable information for establishing sustainable fertilizer management systems to maintain and enhance soil quality. The labile fractions are microbial biomass carbon (MBC), oxidizable organic carbon (OOC), dissolved organic carbon (DOC), particulate organic carbon (POC), hot water extractable carbohydrates (HWC), water extractable carbohydrates (WC).

The soil organic carbon (SOC) content and phosphorous (P) availability in the acid soil is very much reduced because P bounded to humic substances forming a resistant P fraction and also P fix with clay, Fe, and Al contents in acid soil. Soil microorganisms help in P transformation and release from the microbial biomass, solubilisation of inorganic P and mineralization of organic P. The addition of organic materials including manures, crop residues and composts could improve soil P availability and SOC and its fractions (Bhattacharyya et al., 2011). Keeping this in view, an experiment was planned to study the effect of different P levels on the different soil carbon fractions in acid soils.

MATERIALS AND METHODS

Description of study sites

The bulk soil samples for the pot experiment were collected from College of PG Studies in Agricultural Sciences (CPGS-AS) research farm and the experiment was conducted at poly-house of the CPGS that lies between 91° 18′ to 92° 18′ E longitude and 25° 40′ to 26° 20′ N latitude with an altitude of 950 m above sea level. The experimental site falls under subtropical humid climate with high rainfall and cold winter. Monsoon normally sets in the first fortnight of June and remaining active till October with 1000-4000 mm mean annual rainfall and high humidity (above 80%). The maximum temperature rises up to 35°C in the months of July-August and minimum falls down to 5-6°C during the first week of January. The average annual rainfall of the study sites were 2349 mm; mostly confined to May to November and mean daily temperature varied from 2.58°C in January to 32.58°C in August (Choudhury et al. 2012).

Experimental details

The pot experiment was conducted in Completely Randomized Design with five replications and six treatments (Table 1) and French bean was taken as the test crop. Thirty numbers of cylindrical pots having 26 cm height with 25 cm diameter at top and 18 cm diameter at bottom were used in the experiment. Each pot was filled up with 7 kg of dried 2mm sieved soil treated with different treatments. The sources of nitrogen, phosphorous, potassium will be Urea, SSP and MOP respectively. Total amount of phosphorous and potassium as per the treatments were applied at the time of sowing. Nitrogen was applied in three split doses i.e. 50% of RDF at time of sowing, 25% of remaining 50% RDF at 30 DAS, and another 25% at 60 DAS.

Table 1. The treatment details of the experiment

Experimental design	Treatment details
Completely Randomized Design (CRD)	1) T_0- NPK @ 80:0:40 kg ha^{-1}
	2) T_1 - NPK @ 80:20:40 kg ha^{-1}
	3) T_2 - NPK @ 80:40:40 kg ha^{-1}
	4) T_3 - NPK @ 80:60:40 kg ha^{-1}
	5) T_4 - NPK @ 80:80:40 kg ha^{-1}
	6) T_5- NPK @ 80:100:40 kg ha^{-1}

Soil sample collection and processing

The samples were air dried, grinded and passed through a 0.5 mm sieve for determination of organic carbon fractions (Table 2) and immediately stored at 20ºC for analysis of DOC, HWC and MBC (Singh and Lal, 2005). The initial properties of soil were 1.77% SOC, 0.55% OOC1, 1.27% OOC2, 1.77% OOC3, 1.50% POM, 305.73 (µg g^{-1}) MBC, 730.82 (µg g^{-1}) DOC, 74.17 (µg g^{-1}) HWC and 39.73 (µg g^{-1}) WC. The very-labile (OOC1), labile (OOC2) and less-labile (OOC3) fraction of soil carbon were determined using 12N H_2SO_4, 18N H_2SO_4 and 24N H_2SO_4.

Table 2. The parameters and methods for soil organic carbon fractions analysis

Sl. No	Parameter	Method
1.	Oxidisable organic carbon	Modified wet oxidation method (Chan et al., 2001)
2.	Particulate organic matter (POM)	Size-base procedure using sodium hexametaphosphate (Gregorich and Beare, 2008)
3.	Microbial biomass carbon (MBC)	Chloroform fumigation and K_2SO_4 extraction method (Brookes and Joergensen, 2006)
4	Dissolved organic carbon (DOC)	1M KCl extraction followed by 0.45µm polycarbonate filtration method (Zsolinay, 1996 and Mc Dowell et al., 2006)
5.	Hot water extractable carbohydrate	Phenol method (Safarik and Santruckova, 1992)
6.	Water extractable carbohydrate	Phenol method (Safarik and Santruckova, 1992)

RESULTS AND DISCUSSION

Effect of phosphorus levels on different soil carbon fractions at 30 DAS

The soil organic carbon (SOC) content was decreased with mineralization with application of phosphorous (P) and soil microbes obtained energy through the catabolism of soil available C to acquire their necessary nutrients (Sinsabaugh et al., 2013; Sistla and Schimel, 2012) resulting higher microbial carbon fraction (MBC) (Table 3). Fertilization significantly affected microbial community structure (Zhang et al., 2016). The microbial decomposition rate of SOC might depend upon the nutrient limitation and C:N:P ratio (Zhu et al., 2018) (i.e. averaged C:N:P ratio 287:17:1 for soil nutrients and 42:6:1 for soil microbial biomass (Xu et al., 2013)). Microbial activities would be increased with sufficient microbial C:N:P ratio in the nutrients and SOC mineralization stimulated (Wei et al., 2017, 2019). The SOC mineralization increased exponentially with dissolved organic C (DOC):NH4+-N, DOC:Olsen P and microbial biomass (MB)C:MBN ratios (Wei et al., 2020). However there was no significant difference of MBC and particulate organic matter (POM) among the different treatments at 30 days after sowing (DAS) of French bean crop (Table 3). The MBC and particulate organic matter (POM) mineralization in short time period might not be notice. Similar results were reported by (Manna et al., 2006; Lou Y et al., 2011). On contrary, Huang et al. (2009) and Luo et al., (2016) reported that fertilization enhanced particulate organic C (POC) contents.

The result was cleared that the content of SOC and oxidizable organic carbon (OOC) fractions at 30 DAS were significantly decreased with application of increasing doses of P. About 16.67% SOC, 19.09% OOC1, 23.46% OOC2 and 16.67% OOC3 were decreased with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0(NPK @ 80:0:40 kg ha^{-1}). This decrement might be due to microbial translocation, plant uptake and pedogenic process of soil. An optimum C:P ratio was mandatory for mineralization and immobilization of SOC to different C fractionation. The dissolved organic carbon (DOC) ranging from 809.98 to 674.44 µg g^{-1} was also found decreasing with application of increasing dose of P. Approximately 16.73% of DOC was decreasing with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0(NPK @ 80:0:40 kg ha^{-1}). DOC caused C flow and increase in available nutrients and stimulated SOC mineralization. Similarly, Luo et al., (2020) found that the fertilization was significantly decreased the water-soluble organic carbon (WSOC). Fertilization might increased the soil C:N ratio resulting in decreasing DOC. Luo et al., (2020) reported that fertilization increased C:N ratio.

However, the hot water extractable carbohydrates (HWC) and water extractable carbohydrates (WC) were increasing over the T_0. About 5% HWC and 2.4% WC were increased with application of T_5(NPK @ 80:100:40 kg ha^{-1}) over T_0.

Table 3. Effect of phosphorous levels on different soil carbon fractions at 30 DAS old french bean

Treatment	SOC (%)	OOC1 with 12N H_2SO_4 (%) (very-labile)	OOC2 with 18N H_2SO_4 (%) (labile)	OOC3 with 24N H_2SO_4 (%) (less-labile)	MBC (µg g^{-1})	POM (%)	DOC (µg g^{-1})	HWC (µg g^{-1})	WC (µg g^{-1})
T_0	1.86	1.10	1.79	1.86	381.30	1.20	809.98	61.40	34.15
T_1	1.66	0.94	1.64	1.66	382.14	1.18	703.14	56.72	33.24
T_2	1.62	0.95	1.60	1.62	397.17	1.21	700.13	59.90	34.79
T_3	1.54	0.99	1.50	1.54	401.52	1.20	678.17	70.19	34.97
T_4	1.59	1.01	1.55	1.59	397.45	1.19	675.52	63.38	34.61
T_5	1.55	0.89	1.37	1.55	405.07	1.21	674.44	64.53	34.97
Sem±	0.02	0.03	0.03	0.02	0.02	0.02	15.24	2.54	0.41
CD5%	0.08	0.11	0.11	0.07	NS	NS	54.61	9.10	1.47

*OOC-oxidizable organic carbon fraction, MBC- soil microbial biomass carbon, POM- particulate organic matter, DOC- dissolved organic carbon, HWC- hot water extractable carbohydrates, WC- water extractable carbohydrates

Influence phosphorus levels on changing different soil carbon fractions from 30 DAS to crop maturity:

The Fig.1 was indicating the changes of different soil carbon fractions from 30 DAS till crop maturity. The figures of SOC, OOC2 and OOC3 at 30 DAS were noticed in decreasing and also OOC3 at crop maturity was also found decreased. This might be attributed to microbial utilization of such labile fractions of carbon as source of their energy. The SOC and OOC2 at crop maturity were observed depressions and finally gained its content at crop maturity. The POM at 30 DAS and crop maturity were maintained in uniform lined, however, POM at crop maturity was lower than the POM at 30 DAS.

The DOC and MBC at 30 DAS were found higher amount as compared to DOC and MBC at crop maturity. The HWC at 30 DAS and maturity were found not much changed with different P applications. The WC at 30 DAS was little bite higher amount than the WC at crop maturity.

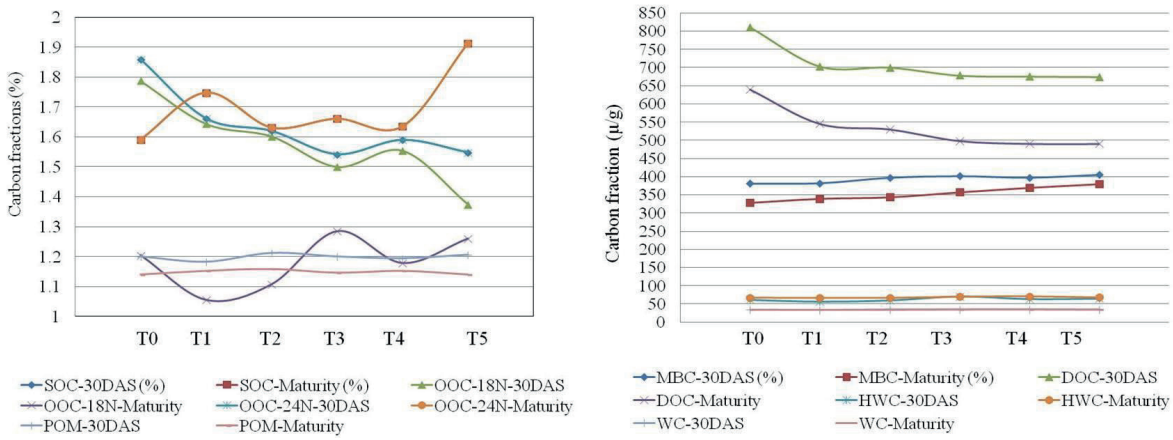

Fig. 1. Effect of phosphorus levels on changing different soil carbon fractions during crop (French bean) growing

CONCLUSION

Application of 100% recommended dose of P_2O_5 (i.e. NPK @ 80:100:40 kg ha^{-1}) in acidic soil was resulted in decreasing about 16.67%, 19.09%, 23.46%, 16.67%, 16.73% of SOC, oxidizable organic carbon fraction 1 (OOC1), OOC2, OOC3 and Dissolved organic carbon (DOC) respectively, however, increased MBC, POM, HWC (5%) and WC (2.4%). Therefore, it was concluded that the mineralization of labile carbon pools such as OOC1, OOC2 and OOC3, DOC were increased the phosphorous availability in acid soil.

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Infestation of yellowtip halfbeak fish, *Hemiramphus marginatus* (Lesueur 1821), a new host of *Nerocila exocoeti* (Crustacea, Isopoda, Cymothoidae) in South East coast of India

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ABSTRACT

The ecto-parasite *Nerocila exocoeti* (Crustacea, Isopoda, Cymothoidae) is reported for the first time from the yellowtip halfbeak fish, *Hemiramphus marginatus* (Teleostei, Beloniformes, Hemiramphidae), collected from Light House Kuppam, Marina Beach, Chennai, South-east coast of India. This was abnormal position on the head surface of host fishes is unique. This black isopod naturally occurs in the marine fish belongs to Exocoetidae family. This is the second time this isopod has been collected in the south coast of India and the first for this host fishes. *Nerocila exocoeti* parasitized 22.55% (15 of 150) of the examined *Hemiramphus marginatus*.

Key words: *Hemiramphus marginatus*, Isopod, *Nerocila exocoeti*, yellowtip halfbeak fish

INTRODUCTION

Parasitic fish diseases constitute one of the most important problems in the fisheries sector. Cymothoids are obligatory fish parasites, infest mostly commercially important fishes. They are protandric hermaphrodites and blood suckers, living on the skin, gill filaments, or in the mouth of the fishes. These parasites retard growth followed by emaciation. Pathological conditions resulting from parasitic diseases assume high magnitude of epidemics under crowded and other unnatural conditions among fish. The order Isopoda includes both terrestrial and aquatic species and represents the second largest order of crustaceans. Isopods are widely distributed in all types of habitats, from terrestrial to marine, fresh and ground waters, and some species are known to be parasitic. Ectoparasitic isopods of the family Cymothoidae inhabit freshwater, brackish and marine environments, utilizing various fish hosts. They may be observed on the body surface, or in the buccal and branchial cavities of the hosts (Trilles, 1969; Brusca, 1981; Ravichandran et al., 2009a; b; Ravichandran et al., 2011; Rameshkumar and Ravichandran 2010, Rameshkumar et al., 2011; Sethi, 2012, Sethi et al., 2013 and Roy et al., 2015). Oktener and Trilles 2004. Report on the Cymothoids (Crustacea, Isopoda) collected from marine fishes in Turkey. Bruce and Harrison-Nelson (1988) reported *Nerocila trichiura*, parasitic on flying fishes of the genera *Exocoetus* and *Cypselurus*. Nerocila is a large genus of the family Cymothoidae including at least 65 species living attached on the skin or on the fins of fishes. As already reported by Trilles (1972, 1979), Williams and Williams (1980, 1981), and Bruce (1987a, b), several species are morphologically highly variable and their identification is often difficult. The variability was particularly studied in *Nerocila armata* and *Nerocila orbignyi* (Monod, 1931), *Nerocila excisa* (Trilles, 1972), *Nerocila sundaica* (Bowman, 1978), *Nerocila acuminata* (Brusca, 1981), *Nerocila arres*, and *Nerocila kisra*

(Bowman and Tareen, 1983), and *N. orbignyi, Nerocila monodi*, and *Nerocila phaiopleura* (Bruce, 1987a).

Unusual attachment of Nerocila exocoeti in Hemiramphus far (Forsskal, 1775) from Parangipettai south east coast of India was reported by Sivasubramanian and Ravichandran during 2015. In this study, we report the occurrence of cymothoid isopods in *Hemiramphus marginatus* for the first time and provide new geographic record for *N. exocoeti* on the south-east coast of India.

MATERIALS AND METHODS

A total of 150 yellowtip halfbeak fish, *Hemiramphus marginatus*, ranging average of 275.8±13.5 mm in length and weight 75.06±11.88 g respectively, were captured from January to December 2011 in Light House Kuppam, Marina Beach, Chennai, South-east coast of India (13º00'N, 80º30' E) and examined for the presence of ecto-parasites. Collected parasites were fixed in 70% ethanol (w/v). Morphological characteristics obtained from collected isopod specimens were used for the identification of parasites according to Pillai (1954), Trilles (1969, 1977 & 1989.), Bruce and Harrison-Nelson (1988). The taxonomic classification of the halfbeak fish host was carried out following Food and Agriculture Organization, FAO sheets (Fischer and Whitehead, 1974) and Fish base (Froese and Pauly, 2008). Sivasubramanian et al., studied during 2011 that the Infestation of *Exocoetus volitans* (Linnaeus, 1758), a new host of *Nerocila exocoeti* (Crustacea, Isopoda, Cymothoidae). Bariche and Trilles (2005) studied in Preliminary check-list of Cymothoids (Crustacea, Isopoda) from Lebanon, parasitizing on marine fishes. New host record, *Parablennius sanguinolentus* (Teleostei, Perciformes, Blenniidae) for *Nerocila bivittata* (Crustacea, Isopoda, Cymothoidae) (Alas et al., 2008). Unusual attachment of *Nerocila exocoeti* in *Hemiramphus far* (Forsskal, 1775) from Parangipettai south east coast of India was reported by Sivasubramanian and Ravichandran during 2015.

RESULTS AND DISCUSSION

Fifteen (22.5%) out of the 150 yellowtip half beak fish *Hemiramphus marginatus* examined specimens were infested with parasitic isopods. The parasites were found on the body surface. Based on morphological characteristics provided below, the isopods were determined as *Nerocila exocoeti* (Fig.1-2). Body surface of the infested fish was abraded due to the infestation. These isopods have occupied much of the head region of the host possibly making discomfort to swim, eat and also other activities of the host fishes. However, some relatively large cymothoids occupy much of the skin tissue. The site of attachment of the parasites on their hosts was also observed.

Prevalence and infestation intensity

The prevalence of infestation of *Nerocila exocoeti* on *H. marginatus* was 22.5% (15 of 150) of the examined fishes. Ten ovigerous females of *N. exocoeti* were collected. Infestation intensity ranged from 1-2 per fish.

Description of female parasite

Body length 25-28 mm, width 7-8 mm body longer than broad, symmetrical, bluish black or steel blue with uniform distribution of chromatophores; cephalon- hemispherical with smoothly rounded anterior margin, posterior border tirsinuate, eyes dark, distinct, set off posteriolateral aspect of cephalon; pleon – distinct narrower than pereon (Fig 3).

Description of male parasite

Body length 10-118 mm, 4-5 width, body very small; eyes dark. Total length of body 2.2-2.9 times maximum width. Body narrower than in female. Head not immersed. Eyes obvious in juveniles and immature males. Pleonites 1 and 2 with ventrolateral margins produced; lateral margins of 15 pleonites not produced.

Geographical distribution

N. exocoeti has a wide range of distribution. It was previously recorded from the Southern India

Fig. 1. *Nerocila exocoeti* attached to the head of yellow tip halfbeak fish, *Hemiramphus marginatus* (lateral view)

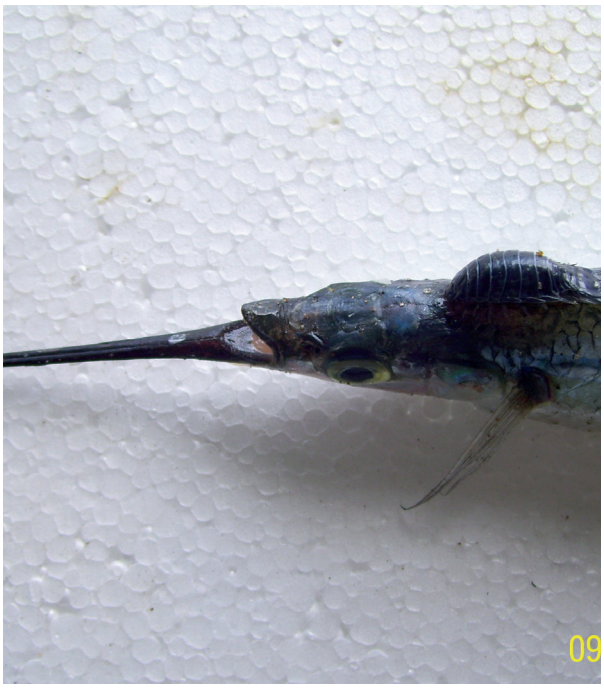

Fig. 2. External lesion due to the infestation of *Nerocila exocoeti*, the damaged skin patch on the head of *Hemiramphus marginatus*

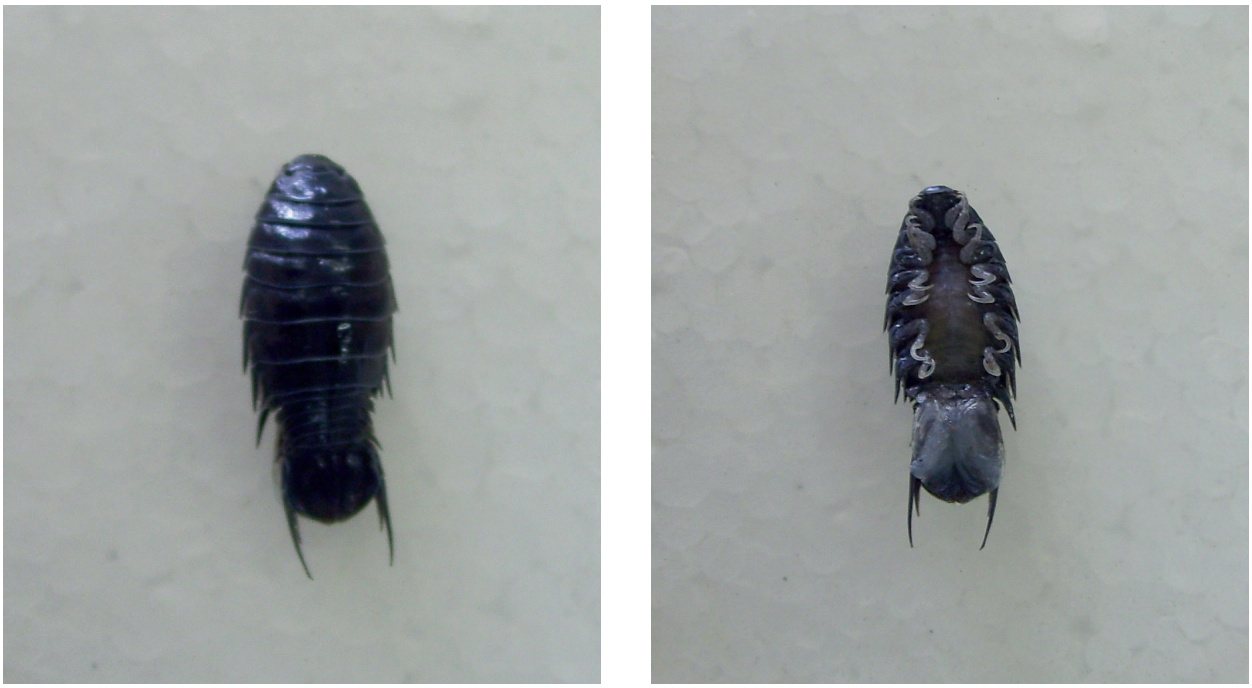

Fig. 3. Dorsal and ventral view of the female of *Nerocila exocoeti* infested on *Hemiramphus marginatus*

(Pillai, 1954) to Papua New Guinea (Trilles, 1977), Indonesia and Taiwan (Bruce and Harrison-Nelson, 1988). The distribution of this species was extended to the Parangipettai, Southeastern coasts of India and also extended to Light House Kuppam, Marina Beach, Chennai, South-east coast of India.

Hosts

The members of family Hemiramphidae are the specific hosts for *N. exocoeti*. Known host spectrum includes *Parexocoetus brachypterus, Cypselurus comatus* (Bruce and Harrison-Nelson, 1988) *Exocoetus volitans* (Sivasubramanian and Ravichandran, 2015) and the range of host fishes is here extended and now includes *Hemiramphus marginatus* (present study).

CONCLUSION

In this study, *N. exocoeti* is recorded for the first time from the *H. marginatus*, together with India, which is a new geographic record. According to its rare occurrence, it is probable that *H. marginatus* might be an occasional or accidental host for this species.

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Population density, nesting ecology and conservation of the Indian giant squirrel (*Ratufa indica*) Erxleben, 1777 in protected areas of Odisha

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ABSTRACT

The population distribution, activity, feeding, ranging and nesting behaviour and conservation aspects of Indian giant squirrel (*Ratufa indica*) across six major habitats in the tropical forests in Eastern Ghats of Odisha are discussed. The species is listed as Least Concern in Red List Data of IUCN and in Schedule I (Part -I) of the Indian Wildlife (Protection) Act, 1972. More than 162 hours were spent in the field survey at different habitats mostly for observing the feeding and activity pattern of Indian giant squirrel including different other wild animal species. Observations were recorded in Budhagiri hills, Karlapat Wildlife Sanctuary (WLS), Sunabeda WLS, Similipal Tiger Reserve (STR), Kuldiha, Kapilash and Lakhari Valley WLS, and proposed Mahendragiri Biosphere Reserve. In total, 38 sightings were recorded from 79 km line transect walk. As per the findings, the Kapilas WLS recorded the high density population i.e. 4.6 squirrels per sq km, whereas Mahendragiri shown the lowest population i.e. 1.2 individuals per sq km. It was inferred that the lowest population density of Indian giant squirrel in Mahendragiri was because of poor canopy cover with low girth evergreen shola forest tree species.

Key words: Eastern Ghats, ecology, feeding, Indian giant squirrel, shola forest

INTRODUCTION

The ecology of squirrels in Asia has been little studied, hindering conservation and management efforts. The Indian giant squirrel or Malabar giant squirrel (*Ratufa indica*) an endemic species to India, is widely distributed from the evergreen to moist and dry deciduous forests of Western and Eastern Ghats and the central Indian hills. It is one of the four species of giant squirrels presently seen in the world. Its populations are severely fragmented due to developmental activities and urbanisation in around forest patched habitats. It is a diurnal large arboreal squirrel completing all life activities over the trees from feeding to breeding. The species is widely distributed in peninsular India (Daniel, 1952; Abdulali and Daniel, 2011; Corbet and Hill 1992) from the evergreen to moist and dry deciduous forests of Western Ghats (Ramachandran 1988; Rout and Swain, 2005), and Eastern Ghats (Kumara and Singh, 2006) and central Indian Hills (Agarwal and Chakraborty, 1979; Datta and Goyal, 1996). The species, like many other squirrels of its genus, is a top canopy dweller, which occasionally comes to the ground (Ramachandran, 1988; Baskaran et al., 2011), mostly to overcome breaks in canopy continuity. The species mostly feeds on seeds, leaves, flowers and bark from trees. It is a solitary living species, constructs

globular nests with leaves and twigs (Baskaran et al., 2011). This animal exhibits 2-3 types of colour pattern with shades of black, brown and dusky white. The body of the rodent varies from deep red to brown with white patches on belly as well as dirty white or cream coloured under parts and forelimbs. The animal has pink lips and nose. Long hairs appear behind the mouth and nose as line moustache. The eyes are coloured in bright dark or light brown. In some individuals the tail length exceeds the length of body from nose to tail. At older age, the tail fur becomes thin and in some individuals it is discontinuous brush with fallen hairs. The powerful and long tail is light brown with creamy white tip. Individuals of both sexes look alike, although females have three set of mammie. It weighs 1.5-2kg and reaches 25-45 cm long and the average life span is round 20 years. These rodents are generally solitary animals, occasionally living in pairs during the mating season (Nameer, 2009). But they stay in social contact with audible calls in patched habitat. These cautious and shy creatures are usually active early in the morning and in the evening. During the midday, they rest in tree holes or large globe-shaped nests in the trees, constructed with twigs and leaves. Each squirrel has 2-5 nests, found within a small territory. One of these nests is made exclusively for reproducing and nursing the young, while others are used as sleeping sites. The Indian giant squirrels are also very agile animals, able to leaps up to 6 meters when travelling among trees. There are 4 sub species of Indian giant squirrels (IGS) in India namely *R. i. indica, R. i. maxima, R. i. centralis, R. i. dealbata* (possibly extinct) and distributed in peninsular India-Eastern Ghats, Western Ghats, Madhya Pradesh,Tamil Nadu, Andhra Pradesh, Karnataka, Odisha, Maharastra (Daniel, 1952).

MATERIALS AND METHODS

Study area

The study was undertaken in selected protected areas of Odisha state during different field visits from 1998-2019. Odisha is located between the parallels of 17.49'N and 22.34'N latitudes and meridians of 81.27'N and 87.29'N longitude and its north western part falls in Chotanagpur Plateau, south western part in Deccan Peninsular and eastern parts fall in east coast of India (Fig. 1).

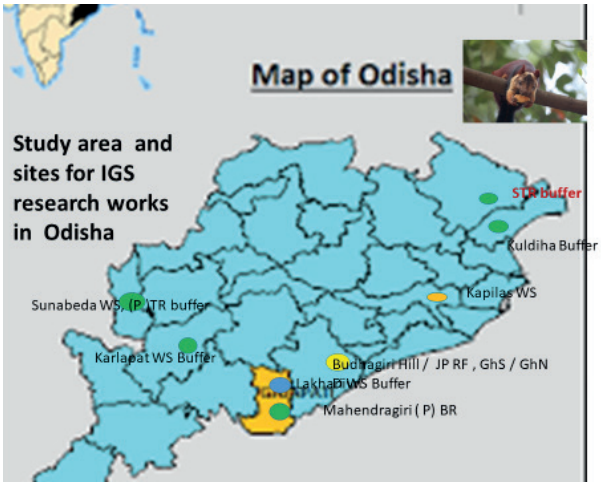
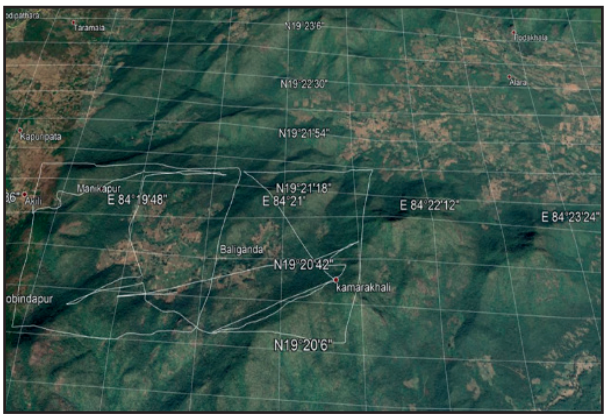


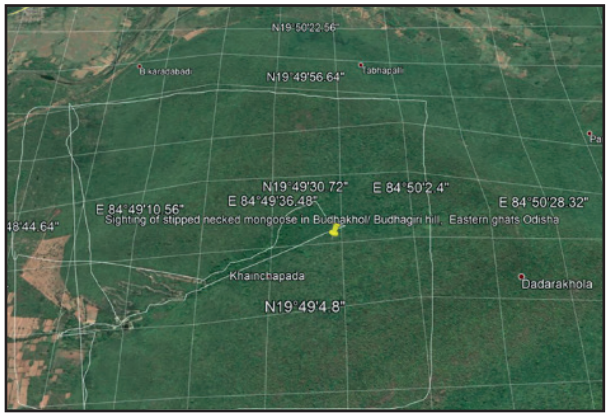
Fig. 1. Map showing the sites where Indian giant squirrel was studied and observation was done

The Eastern Ghat in Odisha extends from part of Ganjam district in eastcoast to Malkangiri in southwest and Nuapada in the west spreading over a vast stretch of ten districts. The study area covers proposed Mahendragiri Biosphere Reserve, Similipal Biosphere Reserve including Karlapat and Lakhari WLS, Budhagiri Hill (Kriamba Reserve Forests) and Pandakhol RF of Ghumusur South forest division, Kotagarh WLS, Kapilas WLS, Kuldiha WLS, some part of Ghumusur North and Karanjia forest division. The study area covers plain forest (70 m) in Ghumusur north division to high mountain peak terrain in Kapilas (535 m), Similipal (1165 m) and in Mahendragiri (1505 m) elevation. Six sites were selected for detailed behavioural data collection on giant squirrels in different habitats, which include moist deciduous forest, dry deciduous forest, river based forest habitats and shola forests (Fig. 2). The vegetation follows a gradient similar to the rainfall, with dry deciduous forests dominating the state followed by semi evergreen patches along hill streams and moist deciduous forests in Similipal and shola forests in Mahendragiri mountain. The state can be divided broadly into four natural divisions: the northern plateau, the Eastern Ghats, the central tract, and the coastal plains. The northern plateau is an extension of the forest-covered Chhotanagpur plateau centred in Jharkhand. The Meghasini peak having altitude of 1165 m is the highest in this region. The Eastern Ghats, extending roughly

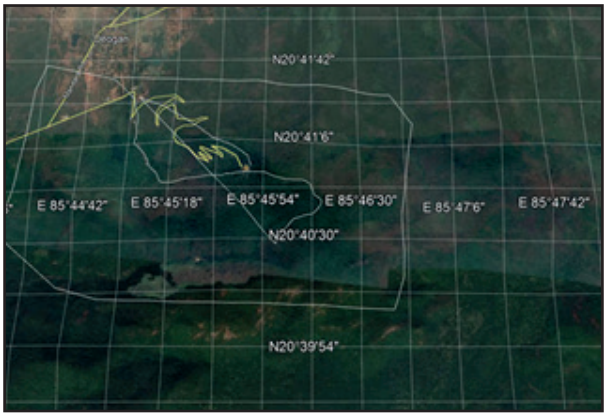
Map 1. Survey area in the Mahendargiri hill keeping the Judhistira temple as centre



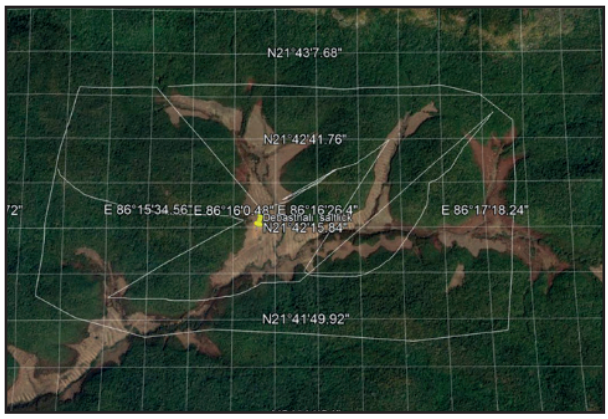
Map 2. Survey area in the Lakhari WLS keeping the Baliganda village as centre



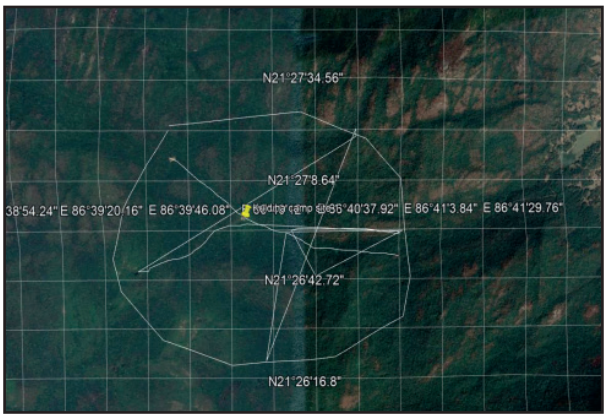
Map 3. Survey area in the Budhagiri hill keeping the Budhakhol shrine area as centre



Map 4. Survey area in the Kapilas hill keeping the Lord Shiva shrine area as centre



Map 5. Survey area in the Debasthali beat house as centre in Similipal Tiger Researve/ Biosphere Reserve



Map 6. Survey area in the Kuldiha Wildlife Sanctuary centred at FRH/ Ecotourism camps

Fig. 2. Maps(1-6) showing the sites where Indian giant squirrels activities were studied and observation: Google maps of survey areas were shown keeping as core of distribution sites in different PAs

parallel to the coast and rising to an elevation of about 3,600 feet (1,100 metres), are remnants of a very ancient line of hills in eastern peninsular India. The central tract is a series of plateaus and basins occupying the inland area to the west and north of the Eastern Ghats. In addition to the Mahanadi, the main rivers are the Subarnarekha, Budhabalanga, Baitarani, Brahmani, Rushikulya and Bahuda. The annual average temperature of the study area ranges from 4ºC at hilly areas to 45ºC at Kapilash forest area with annual average rainfall ranges from 800 mm to 1500 mm (Das and Kar, 2011). The maximum and minimum relative humidity are 88% and 62% respectively. Details of topography and environmental parameters of the study sites have been presented in Table 1.

Survey on distribution of Indian giant squirrel in Odisha

The distribution of giant squirrel were mapped based on the presence and absence, direct sightings, calls and their nests through transect survey of 25 transects covering 79 km laid across different habitat types (Fig. 2). In all the major habitats, an effort was made to sample the river based (along river and stream) microhabitats as they are distinct from surrounding areas in terms of tree species composition and canopy contiguity, especially in the dry deciduous and dry thorn forest. For population density estimation, the line transect method (Burnham et al., 1980) was adopted to estimate population density. In total, 16 transects were sampled with length varying from 2-4 km and were laid systematically covering all the habitats and microhabitats across the sanctuary. The survey was conducted from February 2014 to May 2018. The transects were walked during morning (06:30-10:45 hr) or evening (16:00-18:00 hr) and at every sighting of squirrel we recorded the perpendicular distance, using range finders and group size of the squirrel. Observations on ecological aspects of Indian giant squirrels have been recorded and analyzed (Table 2).

Tools and Techniques

During trail walk, it is contiguous without stop at sighting spot and new individuals are sampled ahead and putting the location, time recorded both in GPS (Garmin 76 Map) and note book. The spotted site was marked and loaded in GPS. The angle and distance of the tree where the animal sighted were calculated from ocular estimation. During later part of the survey i.e. after 2016, the smart phone, Gionee and Motorola were used for google mapping and using digital compass as tools. Canon DSLR 1100D with 200 mm zoom lens was used for photography in field. The free version of Google earth pro is used for identifying the location using map and demarcating the sites.

RESULTS AND DISCUSSION

Extensive survey of the major habitats like tropical moist, dry deciduous and montane sola forest in the sanctuary shows that its distribution is discontinuous and patched in Odisha. The moist and dry deciduous forests with good canopy contiguity with climbers are most preferred place for nesting sites. All the sites are categorised under the forest types of dry deciduous, semi-evergreen and moist deciduous forest patches in Odisha (Champion and Seth, 1968). The presence of animal in high canopy is also detected by collection of call data like frequency, angle of call direction, location in the forest patch, distinguishing different calls by loudness, duration and intensity. In total, 38 sightings were recorded from 79 km line transect walk. Mean group (G) size as 3 with standard error (SE) 0.2 were found based on data where complete counts of individuals were obtained on transects. Population density was estimated using distance-sampling techniques following the software DISTANCE version 6.0 (Buckland et al. 2004). Grouping the data into 20 m perpendicular intervals to the transect laid were calculated. The feeding sites are also located in some isolated mango trees near Jagannathprashad range of Ghumusur north forest division. A lone Indian giant squirrel was found in a moi (*Lannea coromandelica*) tree at Kapilas WLS (Fig. 3). Another IGS was seen near the nest in upper portion of a fig (*Ficus glomerata*) tree in Kapilas WLS (Fig. 4). At Similipal inside the protected area, one pair noticed to come to ground twice which were seen on sal tree at 65ft height. Hence, the population was believed to be in increasing trend. At Kapilas hill range, in another instance, a couple of IGSs, one buck (male) and the other one a doe (female) were

sighted during the courtship at Mahendragiri Reserve forest (Fig. 6). It supports the study that IGS seen in pairs with Nameer, (2009). A male IGS buck was found relaxing over an Indian fig tree at Kapilas hill range (Fig. 5). It was noticed that one squirrel was consuming the flowering pods of jackfruit (*Artocarpus heterophylus*) in its early flowering stage in Budhagiri hills near the Budhakhol shrine at Budhakhol (Kriamba RF, Ghumusur South division). Here in, the animal was observed to move swiftly on fine fig branches and later took complete grip of the branch with support of hind limbs and lower abdomen in a circular manner and holding the pod with the forelimbs in a very fascinating and relaxed manner (Fig. 7, 12). In one case, at Kapilas, one IGS seen jumped from branches of mango tree to collect food. It corroborates with the study uindertaken by Mishra et. al, 1996. In another instance, feeding was noticed in hanging free posture by IGS in Kuldiha WLS (Fig. 11). Indian giant squirrel uses creepers atundi (*Combretum decandrum*) for jumping movement amidst bushy thickets of fig trees, as seen in Kapilas WLS. It was recorded that creepers like siali, atundi acted like a communicating ropeway from tree to tree for swift movement for purposes of mating and escape from predation and disturbances (Fig. 8). Indian giant squirrels are active and agile animals, move from tree to tree taking amazing leaps with limbs spread. The animals are not easy to discover and usually lives alone or in pairs (Mishra et. al.,1996). IGS builds nests in high tree branhes with support from many different creepers. A linear nest of IGS was seen in a tree branch amidst complex of creepers in Kapilas WLS (Fig. 9). A globular nest of IGS was noticed in a tall single tree branches centrally with the support of branches only at Kuldiha WLS (Fig. 10).

Fig. 3. Indian giant squirrel in a Moi (*Lannea coromandelica*) tree at Kapilas WLS

Fig. 4. Indian giant squirrel near the nest in upper portion of a fig (*Ficus glomerata*) tree in Kapilas WLS

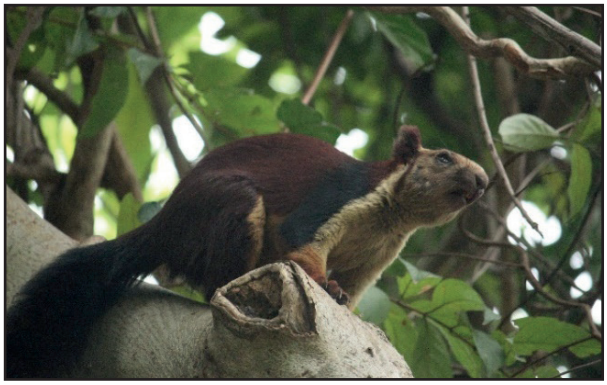


Fig. 5. A male Indian giant squirrel buck over an Indian fig tree at Kapilas.

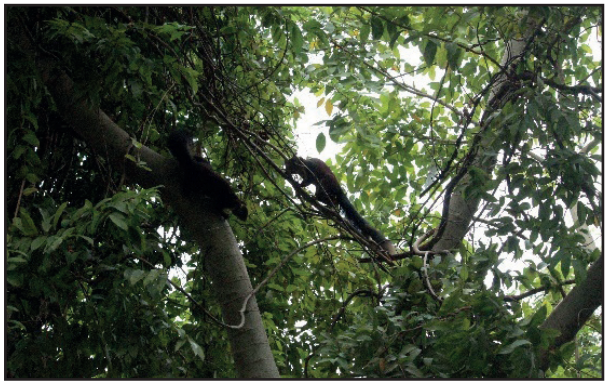


Fig. 6. Indian giant squirrel buck (male) and doe (female) during courtship at Mahendragiri RF

Fig. 7. Indian giant squirrel taking flower pods of jackfruit at Budhakhol shrine area close to temple.

Fig. 8. Indian giant squirrel uses bushy thicket of creepers atundi (*Combretum decandrum*) in Kapilas WLS

Fig. 9. Linear nest of Indian giant squirrel in both tree branch and complex of creepers in Kapilas WLS

Fig. 10. Globular nest of Indian giant squirrel in tall and single tree at Kuldiha WLS

Fig. 11. Feeding in hanging free posture by keeping forearms free to use as arms in Kuldiha WLS

Fig. 12. Movement of IGS on a jackfruit tree at Budhakhol (Kriamba RF, Ghumusur South division)

For population density estimation, different trails and forest roads were used. Results shown that Kapilas was having highlight density population of 4.6 squirrels per sq km and 1.2 individuals per sq km in Mahendragiri. The less number in Mahendragiri was studied to be due to poor canopy cover with low girth evergreen trees. Here, the squirrels come to ground for uncertain reasons like searching of rhizome food in Mahendragiri habitat and chasing movement to female and intruder male by the breeding buck. Daytime activity and feeding patterns were observed consuming more than 62 hours in the field survey altogether in Odisha. The most active part of feeding time is 7.30 hours to 10.45 hours of the spring days. Interim rests were observed during mid-day hour. Sampling revealed that animals take equal time for feeding and resting. The team observed that giant squirrels used seeds, bark, petioles, leaves and fruits from more than 54 plants out of which 3 are large creepers. The identified fodder plants of Indian giant squirrel (*Ratufa indica*) in Kapilash Wildlife Sanctuary was previously studied by Palei et. al. (2017) covering around 65 plant species. The flowers, fruits and soft pulvinus parts of siali creeper were most preferred in Ganjam habitat. In Lakhari habitat, the squirrels prefer karada tree (*Schleichera oleosa*) flower and mango (*Mangifera indica*) twigs in Kapilas area. The squirrel is a top canopy dweller, rarely comes to the ground (Baskaran et al., 2011). It was observed that giant squirrel comes to ground and serves the rhizomes and roots of herbs mostly the roots of lemon grass and takes the orchids over mango tree as food in Mahendragiri habitat. Its home range size varied from 0.6 ha in high density area of south to deer parks in Kapilas to 2.2 ha in low density Lakhari habitat with mean of 1.4 ha in Odisha state. Nesting characteristics assessed through 24 nests surveyed along 79 km transects per trail walks that the squirrel uses around 20 large tree species found, with higher preference to karada and mango. Nests on dhoura and sal trees are significantly far in height, and canopy contiguity may need from nearest non-nest trees, which are attributed to better protection and escape from predators. In Kriamba reserve forest compartment no 4, the density of used and unused nest counting 6 in 100 square meter area. The height of these nests ranges from 6 to 21m. The nesting tree species are teak, piasal, sal, karada and baniyan trees in this reserve forest. There were more fall of fresh broken mango twigs fallen free under the mango trees in Budhagiri area. It was also observed that giant squirrels are active and started feeding during the early sun shine area where as the squirrels remain asleep till 6.39 hrs in cleft areas during winters. In the western part of hill are in a mid-day observation at Lakhari that one giant squirrel climbed high up in to a single non nesting dhaura tree to escape from avian predators like serpent eagle for half an hour. It was noticed that the squirrel sat still without movement under the camouflaged in leafy branches for more than half an hour. After the serpent eagle flew it descended from the high tree and moved further for feeding in other trees. The nests were studied to be composed of 70 leafy twigs. The leafy thick nests are important to hiding the young ones from predation. It was interesting in Kapilas WLS to observe that the squirrels had false dummy nests constructed in around the actual nests in order to camouflage and escape from predators like serpent eagles. The reshus monkeys often disturbed the lower hight nests as they climb to the smaller branch due to their less weight and swift movement than the langurs in Kapilas habitat. However, during an observation a long rectangular nest is located in low canopy of branched small fig (*Ficus glomerata*) tree in Kapilash during march 2019. Maintenance of old nests were also observed as the squirrels used the same nest in Lakhari habitats. Status of giant squirrel (*Ratufa indica*) in Similipal Tiger Reserve was previously studied by Rout et al. (2005) and in Kuldiha the feeding, nesting ecology and conservation of IGS was studied by Nayak and Patra (2015). Previous works were undertaken in Karlapat Wildlife Sanctuary over observation on nesting pattern by IGS in Odisha (Pradhan et al., 2012). The call is a social communication recorded mostly during feeding, breeding and movement

individuals. It was also observed that the call was equally responded for the other ones when the researchers remain in hide out. In Lakhari, the call is recorded from same forest block, from same nesting trees and the call is random at any time during day. Most of the squirrels are concentrated at Medicinal plant conservation site near Chandragiri. They also use a giant baniyan tree (*Ficus bengalensis*) for nest and rest at night and seen around the year at same area ranging from 0.10 to 0.25 sq km. Also they use for chasing the mate in all study areas. It was a rare observation that the animal came to ground and spent very limited time in ground for different activities like communication to different patches of vegetation, chasing movement for mating, in search of rhizome of grasses and herbs as food, and even for drinking water from streams during summer. In Similipal, one pair was photo captured when one on the ground and the other in the climbing posture to a 4ft girth inclined tree trunk during 08.35 hours. It was observed that the giant squirrels crossed the forest road near pillar post (7 km) in compartment 4 of Kriamba RF. It was also observed that they covered about 1 km to the northern stream site for drinking water once a day and come to ground due to break in canopy cover along the forest road.

Table 1. Details of topography and environmental parameters of study sites in Odisha

Sl. No.	Name of the site PAs (WLS/TRs/ BR)	Elevation of obs. place MSL (m)	Average annual rainfall (mm)	Min. winter temp (°C)	Max. summer temp. (°C)	Obs. time for IGS (Hrs.)	Days in PAs	Major bio-geographic regions	Survey transects and IGS seen (km)
1	Similipal TR/ BR	600-1200	Above 1600	5-10	32-37	12	25	Chotnagpur Plateu, EGs	3 nos 10 km
2	Kuldiha WLS	250-450	1400	10-12	37-40	6	4	Eastern Ghats (EGs)	2 nos 6 km
3	Kapilas WLS	120-535	1300	9 - 12	37-42	21	35	Eastern Ghats	3 nos 12 km
4	Budhagiri Kriamba RF	300-600	1500	7 -12	35 -40	28	52	Eastern Ghats	2 nos 8 km
5	Jagannath prashad RF	56-120	1400	10-15	35-44	8	12	Eastern Ghats	1 nos 3 km
6	Kotagarh WLS	300-900	1200	5 -12	32- 38	6	8	Eastern Ghats	3 nos 6 km
7	Karlapata WLS	250-600	1300	8-12	37- 42	8	10	Central tract / EGs	2 nos 4 km
8	Sunabeda WLS	250-450	1200	10-12	37-42	8	24	Decan plateu, EGs	4 nos 8 km
9	Lakhari WLS	250-600	1500	6 -10	37 -40	20	40	Eastern Ghats	2 nos 8 km
10	Mahendragiri RF Pr BR	1200-1505	Above 1700	4 -8	30 -32	7	8	Eastern Ghats	3 no 14 km
	Total 10 sites					118 spent	218 days stay		79 km survey

*WLS- Wildlife Sanctuary, RF/ RFs- Reserve Forests, BR- Biosphere Reserves, TR- Tiger Reserve, EGs- Eastern Ghats.

Table 2. Observations over ecological aspects of Indian giant squirrel in Odisha

SL No	Name of the site PAs (WLS/TRs / BR)	Ground utilisation observed	Observed height of trees where IGS movement seen	Elevation in MSL (m)	Conservation status and population trend
1	Similipal TR/BR	One pair two times comes to ground	Above 65 feet in Sal trees	650 m	Population believed to be in increasing trend
2	Kuldiha WLS	Not observed	overhead 12 feet feeding in jamun tree	245 m	Population believed to be in increasing trend
3	Kapilas WLS	Single individual one times	overhead in Mago tree above 14 feet	110 m	Population believed to be in increasing trend
4	Budhagiri/ Kriamba RF	3 individual crossing the forest road one by one	Seen overhead 10 feetin jack fruit tree	210 m	Population believed to be in decreasing trend
5	Jagannathprashad RF	Not Observed	Moving in mango tree 40 feet above	85 m	Habitat lost and population is pocketed and in decreasing trend
6	Kotagarh WLS	Not Observed	Around 55 feet Sal tree	250 m	Population believed to be in decreasing trend
7	Karlapata WLS	Not Observed	Above 45 feet Dhaura tree	230 m	Population believed to be in increasing trend
8	Sunabeda WLS	Not Observed	Seen overhead above 15 feet	280 m	Population believed to be in decreasing trend
9	Lakhari WLS	Not Observed	Seat silence in Dhaura tree abobe 75 feet	320 m	Population believed to be in decreasing trend
10	Mahendragiri RF/ Pr BR	One for using lemon grass shoot as food	Feeding on Orchids in Mango tree above 12 feet	1335 m	Population was displaced by present road construction and in decreasing trend
11	Badagada range Forests	Not utilising foot hill and plain area.	Only occurs in hill side Tree vegetation	120 m	Habitat synchronized Population believed to be in decreasing trend
12	Pondakhol RF of Soruda Range	Well undergrowths. Ground is not utilized	Distributed in interior patches of vegetation.	450 m	Habitat is best suitable Population; believed to be in increasing trend
13	Ramaguda RF of Berhampur Range	Never come to plains and village side vegetation	Past distributed and use Mango trees for Nests	150 m	Population became isolated, thin and then locally unavailable due to habitat change. Recently vanishing population after 2010

CONCLUSION

Following new findings are recorded during the process of the extensive survey.

i. The Kapilas WLS recorded the high density population i.e. 4.6 squirrels per sq km, whereas Mahendragiri shown the lowest population i.e. 1.2 individuals per sq km.

ii. The Indian giant squirrel live and sustain in high altitude like Mahendragiri. It is recorded that they come to ground for food, chasing of male to male noticed for safeguarding the territory. and also consume the rhizomes of orchids and roots of wild lemon grasses.

iii. Use creepers as support to jump from branches also communicating trees.

iv. Linear, lengthy nests are seen during the course of the study.

v. In one tree, apart from main resting nest, more than one nests are observed. Different cluster of nests are seen in neighbouring trees.

vi. Rearing of neonates made in leafy and thick secretive nests which hunter birds do not recognize and reach.

vii. Monkeys usually disturb IGS and hamper their peaceful leaving just to retain the fruits mostly in mango trees.

viii. They stay in groups during feeding within audible range and communicate with loud vocal calls. The other members respond to alarming calls from lone IGS in danger. Alarming calls are also passed from individual to individual along disturbed areas.

ix. It is seen that they remain calm and quiet and camouflage over tree branches by spreading legs n gripping the branches.

x. In hill ranges, in morning sun shine, they get up and become active and in shed areas they were seen sleeping in the same area at the same time.

xi. In night, they normally do not move

xii. Indian giant squirrel facilitate seed disposals through habit on feeding of fruits and help rejuvenation of the forests and contribute to the ecosystem.

xiii. IGS mostly break branches to collect tender twigs and in the process dropping fallen branches to the ground, those are used by other herbivores like spotted and barking deer as fodder.

xiv. They use different water bodies to drink water once or twice at noon hours or afternoon during hot summer days when human activities are lessened. Sometimes they move a long distance of 500 metres from their nests to fulfil their thirst.

xv. It was studied that mating was exclusively made in the upper branches of tall trees.

CONSERVATION ETHOS

Forest fire, plastic, garbage, tree felling for fuel and branch looping for fodder are major constraints for the habitat restoration and conservation of giant squirrel in non-protected areas. However, there are little threats to giant squirrel populations in major pilgrimage sites like, Kapilas, Budhagiri, Mahendragiri, though poaching is rare as the habitat belongs to sacred and holistic area. Other sites like Kuldiha, Kapilas, Similipal, Lakhari, Karlapat, Kotagarh and Sunabeda are protected areas in Odisha which are under government protection measures. Still there are reports of poaching. Rest of the other distribution areas in Odisha are lack of proper awareness paving the path for more poachings for meat and fur. All these studies give inputs for better food and living towards conserving these scheduled animals. It is a bad practice in southern Odisha that the goat and sheep grazers cut and fell the low girth trees covered with creepers for feed of small animals during grazing in forest land. Rather cutting the entire trees, looping can be a better option to save the fodder tree and continuous supply of fodder branches in different seasons. In Ganjam habitat, the squirrel was practised in fruit collection and hence, people keep them as pet and trained them for their own benefits of procuring mangos, harida (*Terminalia chebula*), bahada (*Terminalia belarica*) and other medicinal fruits and seeds. In central Odisha including the Satkosia Tiger Reserve, siali leaves are major non-timber forest produce (NTFP) items which are regularly collected by locals for their livelihood. The crude siali leaf collectors cut the climbers and drag down the creepers that links different trees suited for giant squirrel movement which should be checked through awareness programs. In few parts IGS habitats in Odisha, tribal people poach the cute animal for easy bush meat and ignorance of the importance of these arboreal animals. In south Odisha, there is still practice of catching the young ones to keep as pets and sometimes sell it for paltry money. In Mayurbhanj, there was an instance of cutting and felling a single tree to poach a giant squirrel that occasionally used it as escape shelter. The killing of these innocent and cute animals should be prohibited. Earlier, due to involvement and awareness by VSS (Vana Surakhya Samiti),

there were significant reduction in anthropogenic pressure like cutting of trees and collection of both siali creeper (*Bauhinia vahlii*) leaves and creepers for binding materials in Budhagiri hill which rejuvenated the vegetation and canopy cover since 2016 sustaining the population of IGS. The need of the hour is to create proper awareness for conservation of this rare and highly endangered animal in non-protected areas of Odisha.

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Avifaunal diversity of National Zoological Park, Delhi

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ABSTRACT

The present study was aimed to prepare a checklist of avifaunal diversity of National Zoological Park (NZP), Delhi. The survey carried out from 20 January 2020 to 30 November 2020. During the survey, a total of 124 species belonging to 52 families were recorded which comprised 69 non-passerine and 55 passerine species. Our efforts resulted in the documentation of accurate data of all the avifaunal species. Based on the collected data highest bird species were found from Muscicapidae (12 species) family, followed by Columbidae (7 species), Anatidae (6 species), Ardeidae (6 species) and Sturnidae (6 species). Black kite (*Milvus migrans*), Common Myna (*Acridotheres tristis*), Jungle babbler (*Turdoides striata*), Indian Peafowl (*Pavo cristatus*), Grey hornbill (*Tockus birostris*), Alexandrine Parakeet (*Psittacula eupatria*), Green pigeon (*Treron phoenicoptera*) and Purple Sunbird (*Nectarinia asiaticus*) were the most abundance and dominant bird species of NZP. The survey data shows that NZP supports a vast variety of avifaunal species diversity. The impressive amount of birds provides ecosystem services, food and aesthetic values for visitor and staffs around NZP. However, the survival of species depends upon the conservation of NZP's natural forest, foraging fauna and artificial ponds.

Key words: Avifaunal diversity, feeding habitat, National Zoological Park, species richness

INTRODUCTION

According to (Ali and Ripley 1987; Manakadan and Pittie, 2004), Indian has 1340 bird species, which is about 13% of the world's birds. Birds are considered as indicator species (Blair, 1993), and they are common denizens of ecosystem. In Delhi, more than 450 species and subspecies have been recorded. The main aim of this survey is to make valuable based line information of the avifaunal species for their future as well as to create awareness for educational purpose and their conservation. National Zoological Park (NZP) bears an impressive amount of avifauna diversity. However, previously no data has been published on the diversity of avifauna species from NZP. The NZP provides habitation for a vast variety of natural as well as artificial flora such as : Common species : *Azadirachta indica, Mangifera indica, Syzygium cumini, Morus rubra, Ficus virens, Eucalyptus hybrida, Alstonia scholaris, Terminalia arjuna, Bauhinia variegata, Ficus religiosa, Cassia fistula, Grevillea robusta, Cassia javanica and Ficus carica.*

Shrubs: *Hibiscus rosa-sinensis, Nerium oleander.*

Herbs: *Phyllanthus urinaria, Gymnema sylvestre* and *Scoparia dulcis.*

Grasses: *Cenchrus ciliaris, Saccharum spontaneum, Cynodon dactylon, Panicum repens and Pennisetum pedicellatum.*

The above habitation is associated with the field survey as a roosting place of the different species of birds at day time. The beat no. 14 serves as the resting place for a Partial Albino Jungle babbler (Khan and Kumar, 2019). And a big pond is

the breeding center of painted stork (*Mycteria leucocephala*) during mid-august to January. In the past few years researchers have studied related to the avifaunal diversity in India (Asomani and Boateng, 2019; Blair, 1993); Biswajit and Parthankar et al., 2016; Chopra et al., 2012; Chopra et al., 2013; Chakdar et al., 2016; Gupta et al., 2009; Harvey, 2003; Lehkar, 2011; Joshi, 2015; Nayan et al., 2005; Pawar et al., 2010. Pawar et al., 2010; Joshi and Shrivastava, 2012; Ramitha and Vijyalaxmi 2001; Sisodia and Moundiotiya, 2005; Verma et al., 2004; Urfi, 2003; NZP supports resident and migratory birds throughout the year. In the past few years significant researchers have studied related to the avifaunal diversity in India (Harvey, 2003 ; Urfi, 2003; Sisodia and Moundiotiya, 2005; Pawar et al., 2010; Joshi and Shrivastava, 2012; Chopra et al, 2012; Chopra et al., 2013; Patil and Hiragond, 2013; Kumar et al., 2016). NZP supports resident and migratory birds throughout the year.

MATERIALS AND METHODS

Study area

The current analysis was observed in the National Zoological Park (28.6044359°N, 77.2461981°E) established in the year 1959. It situated in the middle of Delhi, with an area of 176-acre, a sprawling green island and a motley collection of animals and birds. The Zoo area nestled with artificial ponds, butterfly garden, plant nurseries, and impressive floral and foraging faunal diversity. The entire area is crisscrossed with a good network of concrete roads (Fig. 1).

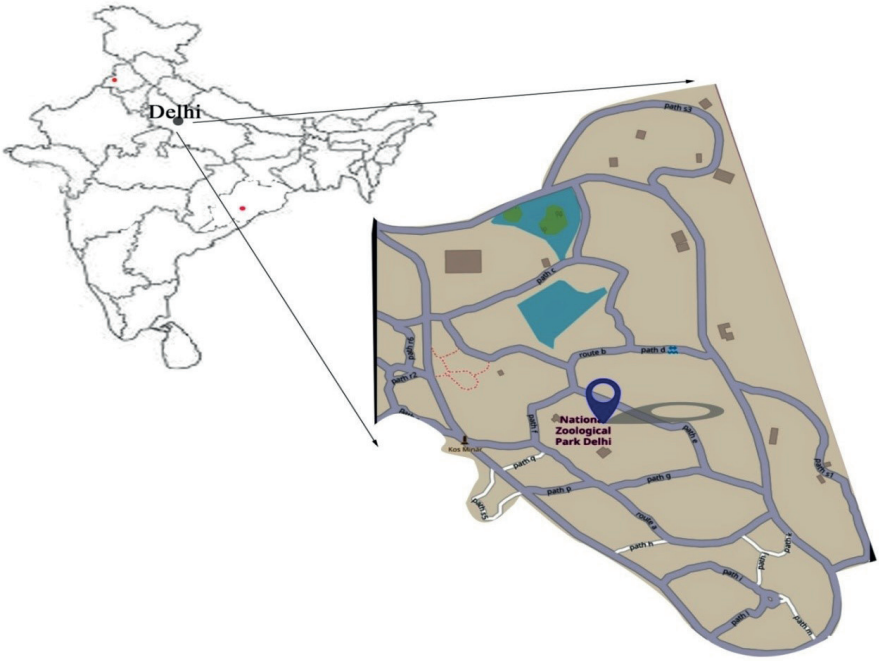

Fig. 1. Map of study area

The surveys were conducted from 20 January 2020 to 30 November 2020 at National Zoological Park to assess the effects of human interferences. Surveys were performed in every parts of study area. Many direct and indirect methods were employed to examine the diversity of avifauna within the study area. In general, for the avifauna that has been actively assessed the authors carried out intensive work during the surveys. The survey was covered both summer (average temperature 25° to 45° C) and winter (average temperature 5° to 25° C) season and habitat. Besides several trapping methods, transects survey, trap station and random walk were established at each sampling site. During study period; regularly fieldwork carried out twice a day in morning (06:30 to 08:30 AM) and in evening

(04:30 to 06:30 PM). All the survey sides were visited at least once in a week. Birds were listed along with the frequency of occurrence for sighting calls or other indirect signs. For the identification, a pocket guide to the Birds of Indian Subcontinent; Ali and Ripley (1999) and Ali and Ripley (1987) were used for sighting and digital data collection. Digital Camera (Nikon D3300 with 70-300 mm zoom lens) and binocular were used for sighting of birds and digital data collection. The scientific names of different bird species were recorded as per the birds of the Indian subcontinent (Manakadan and Pittie, 2004). In the study area, breeding birds' nests were observed and subsequently this information was used to assess the status of birds that are resident to the area or not. For obtaining the percentage of occurrence of families Fig. 2, we used the following formula.

$$\text{Species occurrence (\%)} = \frac{\text{No.of species}}{\text{Total no.of diff. species}} \times 100$$

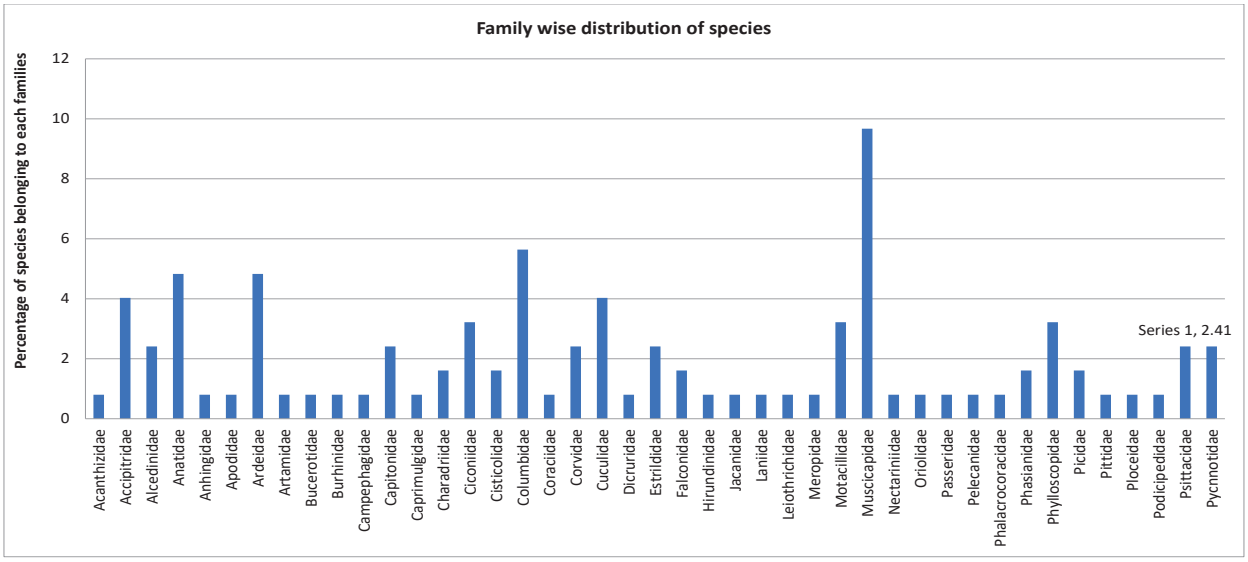

Fig. 2. Percentage distribution of families

RESULTS AND DISCUSSION

On the basis of field survey of NZP, a total of 124 bird species belong to 93 genera and 52 families were recorded. In which 69 are non-passerine and 55 passerine species (Table 1) were recorded.

Out of total 124 species, 31 winter migrants, 12 summer migrants, 5 local migrants and remaining 76 were found resident birds (Fig.3). Out of 76 resident species most of the species have been observed to be positively breeding inside NZP; black kite (*Milvus migrans*), red vented bulbul (*Pycnonotus cafer*), red-wattled lapwing (*Vanellus indicus*), alexandrine parakeet (*Psittacula eupatria*), rose ringed parakeet (*Psittacula krameri*), house crow (*Corvus splendens*), common myna (*Acridotheres stristis*), copper smith barbet (*Megalaima haemacephalaI*), purple sunbird (*Cinnyris asiaticus*), jungle babbler (*Turdoides*

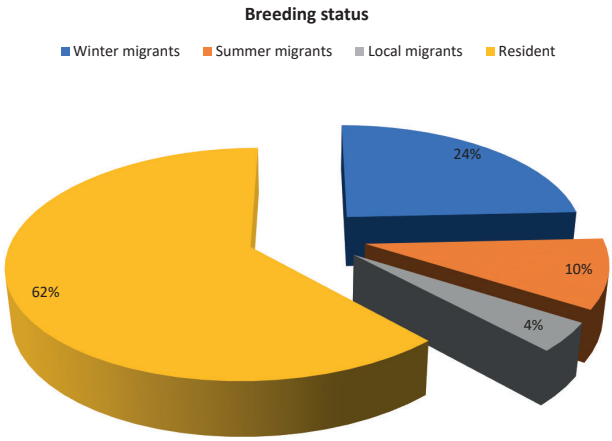

Fig. 3. Distribution of migratory and resident birds

striata) and blue rock pigeon (*Columba livia*) were found common resident. The species richness near the pond (basically water birds) found in impressive

Table 1. Checklist of the birds of National Zoological Park, Delhi

Sl. No.	Family	Common name	Scientific name	Freq-uency	Feeding habitat	Breeding	IUCN status
1.	Acanthizidae	Grey warbler	*Gerygone igata*	C	I	WM	LC
2.	Accipitridae	Black kite	*Milvus migrans*	A	Ca	R,B	LC
3.		Black-winged kite	*Elanus caeruleus*	R	Ca	WM	LC
4.		Crested serpent eagle	*Spilornis cheela*	UC	Ca	#	LC
5.		Indian spotted eagle	*Clanga hastata*	R	Ca	#	VU
6.		Shikra	*Accipiter badius*	A	Ca	R,B	LC
7.	Alcedinidae	Common kingfisher	*Alcedo atthis*	C	P	R,B	LC
8.		Lesser pied kingfisher	*Ceryle rudis*	r	P	R,B	LC
9.		White-breasted kingfisher	*Halcyon smyrnensis*	A	P	R,B	LC
10.	Anatidae	Comb duck	*Sarkidiornis melanotos*	C	P	WM	LC
11.		Common teal	*Anas crecca*	C	P	WM	LC
12.		Pintail duck	*Anas acuta*	C	O	WM	LC
13.		Northern shoveler	*Anas clypeata*	r	O	WM	LC
14.		Spotbilled duck	*Anas poecilorhyncha*	C	G	R,B	LC
15.		Lesser whistling-duck	*Dendrocygna javanica*	A	P	WM	LC
16.	Anhingidae	Darter or snake bird	*Anhinga rufa*	C	P	WM	LC
17.	Apodidae	Asian palm swift	*Cypsiurus balasiensis*	A	I	R	LC
18.	Ardeidae	Little egret	*Egretta garzetta*	A	P	SM	LC
19.		Large egret	*Casmerodius albus*	C	P	R	LC
20.		Cattle egret	*Bubulcus ibis*	A	P	R,B	LC
21.		Median egret	*Mesophoyx intermedia*	A	I	R	LC
22.		Indian pond-heron	*Ardeola grayii*	A	P	R,B	LC
23.		Night heron	*Nycticorax nycticorax*	C	P	R	LC
24.	Artamidae	Ashy woodswallow	*Artamus fuscus*	UC	F	LM	LC
25.	Bucerotidae	Grey hornbill	*Tockus birostris*	A	F	R	LC
26.	Burhinidae	Eurasian stone-curlew	*Burhinus oedicnemus*	r	I	WM	LC
27.	Capitonidae	Blue throated barbet	*Megalaima asiatica*	C	F	R	LC
28.		Brown headed barbet	*Megalaima zeylanica*	A	F	R,B	LC
29.		Coppersmith barbet	*Megalaima haemacephala*	A	F	R,B	LC
30.	Caprimulgidae	Indian nightjar	*Caprimulgus asiaticus*	C	In	R	LC
31.	Campephagidae	Large cuckooshrike	*Coracina macei*	UC	Ca	LM	LC
32.	Charadriidae	Red-wattled lapwing	*Vanellus indicus*	A	I	R,B	LC
33.		Yellow-wattled lapwing	*Vanellus malabaricus*	r	I	WM	LC
34.	Ciconiidae	Black necked stork	*Ephippiorhynchus asiaticus*	UC	P	LM	NT
35.		Painted stork	*Mycteria leucocephala*	A	P	LM	NT

Sl. No.	Family	Common name	Scientific name	Freq-uency	Feeding habitat	Breeding	IUCN status
36.	Ciconiidae	White stork	*Ciconia ciconia*	UC	Ca	WM	LC
37.		White necked stork	*Ciconia episcopus*	UC	Ca	WM	VL
38.	Cisticolidae	Ashy wren warbler	*Prinia socialis*	r	I	WM	LC
39.		Jungle prinia	*Prinia sylvatica*	r	I	WM	LC
40.	Columbidae	Blue rock pigeon	*Columba livia*	A	G	R,B	LC
41.		Domestic pigeon	*Columba livia domestica*	C	G	R,B	LC
42.		Indian ring dove	*Streptopelia decaocto*	C	G	R	LC
43.		Little brown dove	*Streptopelia senegalensis*	UC	G	R	LC
44.		Red turtle dove	*Streptopelia tranquebarica*	UC	G	R	LC
45.		Spotted dove	*Streptopelia chinensis*	A	G	R	LC
46.		Yellow-legged green-pigeon	*Treron phoenicoptera*	C	F	R	LC
47.	Coraciidae	Indian roller	*Coracias benghalensis*	C	I	R	LC
48.	Corvidae	Rufous treepie	*Dendrocitta vagabunda*	A	I	R,B	LC
49.		House crow	*Corvus splendens*	A	O	R,B	LC
50.		Jungle crow	*Corvus macrorhynchos*	A	O	SM	LC
51.	Cuculidae	Asian koel	*Eudynamys scolopacea*	A	F	R	LC
52.		Common cuckoo	*Cuculus canorus*	UC	I	R	LC
53.		Common hawk-cuckoo	*Hierococcyx varius*	UC	I	R	LC
54.		Greater coucal	*Centropus sinensis*	A	O	WM	LC
55.		Pied – crested cuckoo	*Clamator jacobinus*	r	O	SM	LC
56.	Dicruridae	Black drongo	*Dicrurus macrocercus*	A	I	R,B	LC
57.	Estrildidae	Scaly -breasted munia	*Lonchura punctulata*	UC	I	R,B	LC
58.		White throated munia	*Lonchura malabarica*	r	G	R	LC
59.		Red munia	*Estrilda amandava*	C	I	R	LC
60.	Falconidae	Red-necked falcon	*Falco chicquera*	r	Ca	WM	NT
61.		Peregrine falcon	*Falco peregrinus*	r	Ca	WM	LC
62.	Hirundinidae	Barn swallow	*Hirundo rustica*	R	I	WM	LC
63.	Jacanidae	Pheasant-tailed jacana	*Hydrophasianus chirurgus*	r	Ca	WM	LC
64.	Laniidae	Bay backed shrike	*Lanius vittatus*	r	Ca	R	LC
65.	Leiothrichidae	Large grey babbler	*Argya malcolmi*	UC	O	R	LC
66.	Meropidae	Green bee-eater	*Merops orientalis*	UC	I	R	LC
67.	Motacillidae	Grey wagtail	*Motacilla cinerea*	C	I	WM	LC
68.		Large pied wagtail	*Motacilla maderaspatensis*	UC	I	WM	LC
69.		Yellow headed wagtail	*Motacilla citreola*	UC	I	WM	LC
70.		White wagtail	*Motacilla alba*	A	I	WM	LC

Sl. No.	Family	Common name	Scientific name	Freq-uency	Feeding habitat	Breeding	IUCN status
71.	Muscicapidae	Bluethroat	*Luscinia svecica*	UC	I	WM	LC
72.		Black redstart	*Phoenicurus ochruros*	UC	I	WM	LC
73.		Common babbler	*Turdoides caudatus*	A	I	R	LC
74.		Indian robin	*Saxicoloides fulicata*	A	I	R	LC
75.		Jungle babbler	*Turdoides straita*	A	I	R	LC
76.		Jungle babbler (albino)	*Turdoides straita*	r	I	R	LC
77.		Magpie robin or dhyal	*Copsychus saularis*	A	I	R	LC
78.		Siberian rubythroat	*Luscinia calliope*	UC	I	WM	LC
79.		Spotted flycatcher	*Muscicapa striata*	UC	I	LM	LC
80.		Streaked faintail warbler	*Cisticola juncidis*	A	I	R	LC
81.		Common tailorbird	*Orthotomus sutorius*	A	I	R	LC
82.		Yellow-eyed babbler	*Chrysomma sinense*	UC	F	R	LC
83.	Nectariniidae	Purple sun bird	*Cinnyris asiaticus*	A	N	R	LC
84.	Oriolidae	Eurasian golden oriole	*Oriolus oriolus*	C	I	SM	LC
85.	Passeridae	House sparrow	*Passer domesticus*	r	I	R	LC
86.	Pelecanidae	White or rosy pelican	*Pelecanus onocrotalus*	UC	P	R,B	LC
87.	Phalacrocoracidae	Little cormorant	*Phalacrocorax niger*	A	P	SM	LC
88.		Indian cormorant	*Phalacrocorax fuscicollis*	C	P	SM	LC
89.	Phasianidae	Grey francolin	*Fracolinus pondicerianus*	UC	O	WM	LC
90.		Indian peafowl	*Pavo cristatus*	A	O	R,B	LC
91.	Phylloscopidae	Common chiffchaff	*Phylloscopus collybita*	UC	I	WM	LC
92.		Dusky warbler	*Phylloscopus fuscatus*	UC	O	R	LC
93.		Greenish warbler	*Phylloscopus trochiloides*	C	I	R, B	LC
94.		Hume's leaf warbler	*Phylloscopus humei*	UC	I	WM	LC
95.	Picidae	Black-rumped flameback	*Dinopium benghalense*	C	O	R,B	LC
96.		Yellow-crowned woodpecker	*Leiopicus mahrattensis*	UC	I	R,B	LC
97.	Pittidae	Indian pitta	*Pitta brachyura*	UC	I	#	LC
98.	Ploceidae	Baya weaver	*Ploceus philippinus*	UC	I	SM	LC
99.	Podicipedidae	Little grebe	*Tachybaptus ruficollis*	C	P	R,B	LC
100.	Psittacidae	Alexandrine parakeet	*Psittacula eupatria*	A	F	R,B	NT
101.		Plum headed parakeet	*Psittacula cyanocephala*	A	F	R	LC
102.		Rose ringed parakeet	*Psittacula krameri*	UC	F	R,B	LC

Sl. No.	Family	Common name	Scientific name	Freq-uency	Feeding habitat	Breeding	IUCN status
103.	Pycnnotidae	Red-vented bulbul	*Pycnonotus cafer*	A	F	R,B	LC
104.		Red-whiskered bulbul	*Pycnonotus jocosus*	A	F	R,B	LC
105.		White-cheeked bulbul	*Pycnonotus leucotis*	UC	F	SM	LC
106.	Rallidae	Common moorhen	*Gallinula chloropus*	C	I	R,B	LC
107.		Eurasian coot	*Fulica atra*	C	O	R,B	LC
108.		White-breasted waterhen	*Amaurornis phoenicurus*	C	I	R,B	LC
109.	Recurvirostridae	Black-winged stilt	*Himantopus himantopus*	r	I	SM	LC
110.	Scolopacidae	Common sandpiper	*Actitis hypoleucos*	C	I	WM	LC
111.		Green sandpiper	*Tringa ochropus*	C	P	WM	LC
112.	Strigidae	Oriental scops owl	*Otus sunia*	#	Ca	R	LC
113.		Spotted owlet	*Athene brama*	C	Ca	R	LC
114.	Sturnidae	Bank myna	*Acridotheres ginginianus*	A	I	R	LC
115.		Brahminy starling	*Sturnus pagodarum*	C	I	R	LC
116.		Common myna	*Acridotheres tristis*	A	O	R	LC
117.		Jungle myna	*Acridotheres fuscus*	r	O	R	LC
118.		Pied starling	*Gracupica contra*	A	I	R	LC
119.		Rosy starling	*Pastor roseus*	r	I	R	LC
120.	Threskiornithidae	Black-headed ibis	*Threskiornis melanocephalus*	C	P	SM	NT
121.		Red-naped ibis	*Pseudibis papillosa*	C	O	SM	LC
122.	Tytonidae	Barn owl	*Tyto alba*	UC	Ca	R	LC
123.	Upupidae	Eurasian hoopoe	*Upupa epops*	C	I	R,B	LC
124.	Zosteropidae	Indian white eye	*Zosterops palpebrosus*	C	I	R,B	LC

Abbreviations: LC – Least concern, NT- Near Threatened, V – Vulnerable, R- Resident, WM- Winter Migrant, SM- Summer Migrant, LM- Local migrant Ca- Carnivores, F-Frugivores, G-Granivores, I- Insectivores, N-Nectarivores, O-Omnivores, P- Piscivores, #- Recorded only once, A- Abundant, C- Common, UC- Uncommon r- Rare, B- Breed.

amount. black kite (*Milvus migrans*), jungle babbler (*Turdoides striata*) and peafowl (*Pavo cristatus*) showed the maximum species richness in NZP as compare to other resident birds. black headed ibis (*Threskiornis melanocephalus*) mostly sighted inside Indian rhinoceros (*Rhinoceros unicornis*) enclosure. The NZP official provides good food and shelter for migratory as well as resident birds. Out of 124 bird species, 5 species were found Near Threatened (NT), 2 species were Vulnerable (V) and remaining all were found Least Concern (LC) under the IUCN Red List of Threatened Species (2017).

The species richness and abundance of avifauna is due to more floral diversity which provides impressive amount of food as well as nesting and breeding sites. Birds play a vital role and are key species in agriculture ecosystem to maintain the ecological balance, they provide important ecosystem services; pest control, pollination, seed dispersal. The NZP area birds are composed of insectivores (52) followed by piscivores (20), frugivores (14), omnivores (15), carnivores (14), granivores (8) and only 1 species of nectarivores (Fig.4).

Table 2. Avifauna represented in families

Sl. No	Family	Percentage occurrence
1	Acanthizidae	0.80
2	Accipitridae	4.03
3	Alcedinidae	2.41
4	Anatidae	4.83
5	Anhingidae	0.80
6	Apodidae	0.80
7	Ardeidae	4.83
8	Artamidae	0.80
9	Bucerotidae	0.80
10	Burhinidae	0.80
11	Campephagidae	0.80
12	Capitonidae	2.41
13	Caprimulgidae	0.80
14	Charadriidae	1.61
15	Ciconiidae	3.22
16	Cisticolidae	1.61
17	Columbidae	5.64
18	Coraciidae	0.80
19	Corvidae	2.41
20	Cuculidae	4.03
21	Dicruridae	0.80
22	Estrildidae	2.41
23	Falconidae	1.61
24	Hirundinidae	0.80
25	Jacanidae	0.80
26	Laniidae	0.80
27	Leiothrichidae	0.80
28	Meropidae	0.80
29	Motacillidae	3.22
30	Muscicapidae	9.67
31	Nectariniidae	0.80
32	Oriolidae	0.80
33	Passeridae	0.80
34	Pelecanidae	0.80
35	Phalacrocoracidae	0.80
36	Phasianidae	1.61
37	Phylloscopidae	3.22
38	Picidae	1.61
39	Pittidae	0.80
40	Ploceidae	0.80
41	Podicipedidae	0.80
42	Psittacidae	2.41
43	Pycnnotidae	2.41
44	Rallidae	2.41
45	Recurvirostridae	0.80
46	Scolopacidae	0.80
47	Strigidae	1.61
48	Sturnidae	4.83
49	Threskiornithidae	1.61
50	Tytonidae	0.80
51	Upupidae	0.80
52	Zosteropidae	0.80

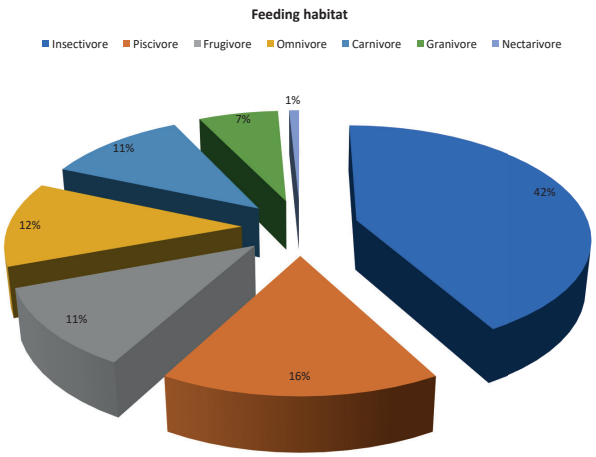

Fig. 4. Distribution of feeding habitat of NZP birds

A good percentage of insectivore bird species shows that the NZP has good insect species. Accidently the state bird of Delhi; house sparrow (*Passer domesticus*) were found only once in this year in front of NZP gate. Surprisingly every year gray wagtail (*Motacilla cinerea*) comes during winter, but this year we first sighted on 1st September 2020 inside the swamp deer enclosure. The results of the study could provide baseline information that would be helpful in understanding the avifauna species diversity and their distribution patterns. Awareness and conservation programs are also needed in order to understand the importance of bird species and their significant contribution to maintain the ecosystem.

CONCLUSION

The present survey provides the prelusive framework to understand the avifaunal diversity of National Zoological Park, Delhi. It indicates that even in metropolitan cities like Delhi still selected habitats have supported a significant number of avifauna. Hence, it is concluded that in order to attract more and more birds, it needs to plant all season varieties of flowering plants, fruiting trees facilitating nesting sites to avifaunal species. Therefore, it is recommended that regular monitoring of the survey site should be carried out every year and every season with support from university faculties and students to protect the marvelous and sustainable ecosystem like NZP to ensure the better protection of natural resources to ensure sustainable ground for the welfare of birds.

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Geo-spatial analysis of land use and land cover in Bhitarkanika Wildlife Sanctuary, Odisha

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ABSTRACT

Mapping and monitoring of land use land cover (LULC) changes is vital for sustainable development, planning and management. Based on Remote Sensing (RS) and Geographic Information System (GIS) techniques, the study is an attempt to monitor the changes in LULC patterns of Bhitarkanika Wildlife Sanctuary in Kendrapara District of Odisha for the period of 30 years from 1990 to 2020. The total area of the Bhitarkanika Sanctuary is taken under study with an area of 672 km^2. This study aimed to calculate changes in LULC within the period of 30 years with an interval of 10 years respectively i.e. 1990-2000, 2000-2010 and 2010-2020 and satellite data were used to identify the change. The use of geo-informatics has enabled us to assign spatial connotations to land use land cover changes namely, population pressure, climate, terrain, etc., which drive these changes. This has helped scientists to quantify these tools and to predict various scenarios. However, Landsat Satellite images adequately aided to detect changes brought down by man or nature in due course of time in three decades which was analysed for the study area. The study aims to detect the change in different land use land cover categories within the Bhitarkanika Wildlife Sanctuary. Ancillary data and local expert knowledge were necessary to expose long-term trends and formulate explanations in a region that surrounds and includes a sanctuary which have been largely devoid of significant direct anthropogenic impact. The reasons for such changes could generally be explained with detailed field-based data sets and such information does not exist at the requisite spatial and temporal scales. Remote sensing datasets, e.g., Landsat imagery, provided the only feasible method to enumerate the trends in land use and land cover in the spatially extensive study area. The changes in the pre and post classification maps were performed by comparison of area based on different land use classes and using change matrix analysis. The result highlighted the changes in the spatial extent of the mangroves and other land use categories in the study areas as a result in due course of time. The present study also deals with periodic assessment and monitoring of the mangroves of Bhitarkanika Sanctuary.

Key words: GIS, land use and land cover, mangroves, mud flat, remote sensing, satellite imageries

INTRODUCTION

Land use and land cover changes, apart from changing the physical dimension of the spatial extent of the land use and land cover classes, also influence many of the secondary processes which lead to the eventual degradation of the ecosystems of the earth (Dregne and Chow, 1992). Land cover refers to the physical and biological cover over the surface of land, including water, vegetation, bare soil and/or artificial structures (Ellis et al., 2007). Land-use/cover is largely determined by the ecological conditions, altitudes, geological structure and slope along with technological, socio-economic and institutional set-up, which also influences the land-use pattern (Rai et al., 1994). Changes in the condition and composition of land-cover affects climate, bio-geochemical cycles, energy fluxes and livelihoods of people (Vitousek et al., 1997; Duong et al., 2016; Gashaw et al., 2018). With the increase in population and urbanization, large areas of forest covers are being converted into other land use categories.

MATERIALS AND METHODS

Bhitarkanika in Odisha is the deltaic region formed by the alluvial deposits of sacred river Brahmani, Baitarani and Dhamra and happens to be a unique habitat with mangrove vegetation on the sides of meandering creeks and tidal mudflats (Fig. 1). The mangrove forests of Bhitarkanika Wildlife Sanctuary constitute and are largely confined between 86° 45′ E to 87° 50′ E longitudes and 20° 40′ N to 20° 48′ N latitudes. In the year 1975, Bhitarkanika was declared as a Wildlife Sanctuary under the Wildlife (Protection) Act, 1972 with an area of 672 km^2. Considering the ecological, faunal, floral, geomorphological and zoological importance and for the purpose of protecting, propagating and developing wildlife and its environment, Govt. of Odisha declared Bhitarkanika as a National Park on 3rd October 1988 with an area of 145 km^2. Average annual rainfall is around 1600 mm. Temperature varies from a minimum of 10°-15° C in December-January to maximum 40°-45° C in May-June. It is one of the largest mangrove ecosystems of India. Bhitarkanika is the home to diverse flora and fauna out of which some are endemic. It is the ideal habitat for the reptiles like estuarine crocodile, water monitor lizard, king cobra and python (Chadha and Kar, 1999). Important avifauna includes the kingfishers, storks, ibises, waders and varieties of migratory ducks like bar headed goose, brahminy duck, gadwall, pintail, etc. Fauna such as leopard cat (*Prionailurus bengalensis*), fishing cat (*Prionailurus viverrinus*), jungle cat (*Felis chaus*), hyeana (*Hyaena hyaena*), jackal (*Canis aureus*), sambar (*Rusa unicolour*), cheetal (*Axis axis*), rhesus macaque (*Macaca mulatta*), wild pig (*Sus scrofa*), small indian civet (*Viverricula indica*), rattel (*Mellivora capensis*), porcupine (*Hystrix indica*), water monitor lizard (*Varanus salvator*) smooth coated otter (*Lutrogale perspicillata*), etc. were also recorded from this study area (Mohanty et al., 2004).

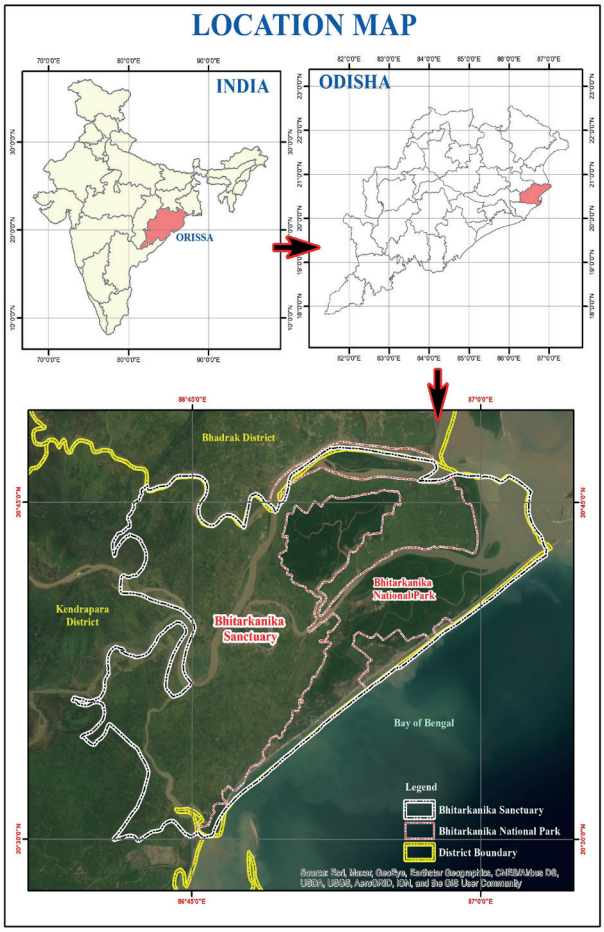


Fig. 1. Location map of Bhitarkanika Wildlife Sanctuary, Odisha, India

Analysis of data

Multi-resolution satellite data over the study area, such as Landsat MSS, Landsat TM, Landsat ETM+ and Landsat OLI were acquired from USGS Earth Explorer portal for the period 1990 to 2020. Four different years data starting from 1990, 2000, 2010 and 2020 were taken into account for the analysis. The dates were chosen in the long dry season in order to use images that were sensed in similar conditions and similar time for a coherent data analysis (Konko et al., 2020). Level 1 product of Landsat archive of USGS was downloaded. All images are ortho-rectified and the details regarding satellites, their acquisition dates and times are listed in Table 1.

Table 1. The satellite data showing year, date, time and resolution

Year	Satellite	Date of pass	Time (UTC)	Resolution	Path/row
1990	Landsat 4 TM	18-Dec-1990	03:54	30 m	139/046
2000	Landsat 7 ETM+	29-Mar-2000	04:30	30 m	139/046
2010	Landsat 7 ETM+	20-Jan-2010	04:29	30 m	139/046
2020	Landsat 8 OLI	28-Mar-2020	04:38	30 m	139/046

The requirement of the study defines the scale at which the mapping is to be done so that details can be extracted from the remote sensing data (Nayak and Bahuguna, 2001). For the current study, Landsat data of 30 mts. Resolution has been taken of four time periods starting from 1990 to 2020, with a span of 30 years having an interval of ten year each. Landsat Thematic Mapper 1990 has been used as the base map for the classification. All the satellite images were taken in the dry season to get the same landscape of the study area throughout the time period. Also getting satellite images of same seasons is considered to be ideal to avoid confusion while mapping. It is well known that season wise, the satellite images change and the landscape also gives different signature on colour, tone, texture of the same feature. The data set obtained for all the four years were first geometrically corrected. Digital image processing technique has been adopted for classification of land use and land cover classes occurring in Bhitarkanika Wildlife Sanctuary area. Raw Landsat images of four different years were taken for band composition and generation of FCC (False Colour Composites) images.

Survey of India toposheets was also geo-rectified and mosaiced to study the land use change detection and then geo-registered to UTM (Universal Transverse Mercator) projection of WGS (World Geodetic System) 84 datum. Re-sampling of satellite images were carried out using ERDAS IMAGINE 2013. The resolution was kept in the re-sampling process at 30 meter for analysis. The visual image interpretation technique was adopted to classify the land use and land cover.

All the database was then exported so as to be used in ERDAS IMAGINE 2013 for layer stacking after which the FCC (False Colour Composite) of each images are generated. After which each geo referenced satellite images are exported and was used in ARC GIS 10.7 for further land use analysis. The satellite images of all the time period were mosaiced on top of the base data and the boundary of Bhitarkanika Wildlife Sanctuary was manually digitized from the Survey of India Toposheets (OSM Series) with UTM WGS 84 projection to get the study area. As it is to be mentioned that the total area of Bhitarkanika Wildlife Sanctuary is 672 km^2 as per Govt. of Odisha vide the notification No. 6958/FFAH Dt. 22.4.1975, to be declared as Bhitarkanika Wildlife Sanctuary and an area of 145 km^2 was declared as a National Park Vide Notification No.19686 / Forest and Environment Deptt./ Dated 16.9.98 to be declared as Bhitarkanika National Park which is the core area of the existing Sanctuary (Chadha and Kar, 1999).

All the Landsat images are provided with ortho- rectification with UTM projection and WGS 84 datum by USGS. Therefore, the existing

geo-rectification of all the images are used and not re-rectified for this study. Landsat collections Level-1 data products consists of quantized and calibrated scaled Digital Numbers (DN) representing the multispectral image data. The Digital Numbers (DN) of each Landsat images were rescaled to top of atmosphere (TOA). To discriminate of aquatic and terrestrial areas from satellite imagery, Normalized difference water index (NDWI) was used because if its efficiency and convenience. NDWI is computed according to the Equation

$$\text{NDWI} = \frac{\text{Green} - \text{Near Infra Red } (^{*}\text{NIR})}{\text{Green} + \text{Near Infra Red } (^{*}\text{NIR})}$$

*NIR = Near Infra Red, Green band.

NDWI seeks to maximize reflectance if water using green wave lengths, minimize the low reflectance of NIR by water features and finally, take advantage if high reflectance of NIR by both the vegetation and soil. Typically, as a result water has a positive value while soil and vegetation have either nil or negative values.

Field verification was first carried out in order to know the ground truth. The visual interpretation of the satellite data is difficult without the field verification and training data sets which is later matched with the remote sensing image to do the final classified maps. The images were carefully classified and interpreted on the basis of tone, texture, colour, pattern, etc. The current classification is done using ArcGIS 10.7, a supervised classification was run. The analysis was again refined by coding through visual interpretation to generate the required 9 classes. The references for verification were SOI Toposheets, High resolution satellite imagery

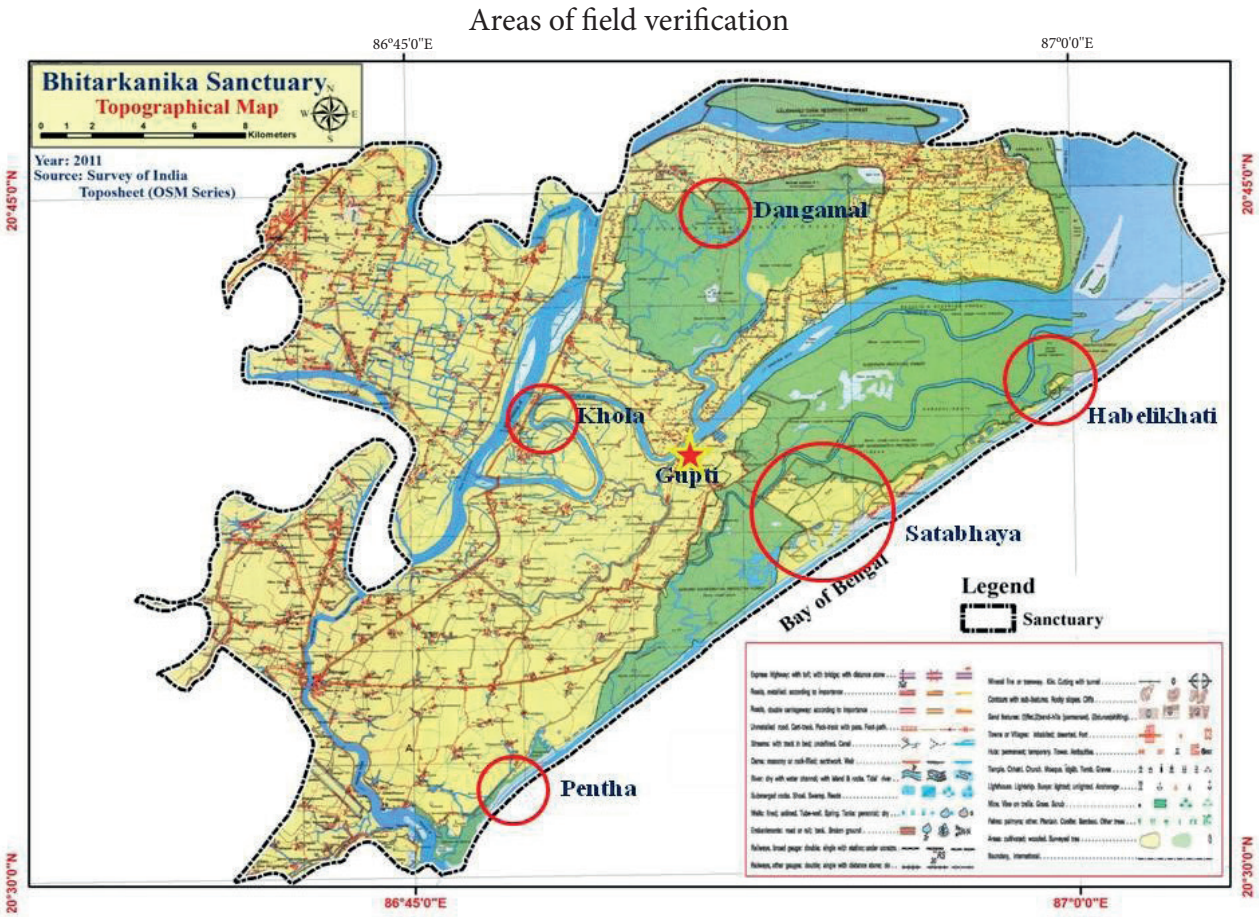


Fig. 2. Areas of field verification in Bhitarkanika Wildlife Sanctuary, Odisha

from online ArcGIS, Google Pro and dedicated field survey. The field verification was basically conducted to know the actual ground reality.

In the process of land use and land cover analysis, the Ground Control Points (GCPs) identified for field verification where the ground truth was conducted, the field truth collected from sanctuary area was taken in account and the colour, tones, textures of the images were also considered for interpretation. River, Mudflats, Sandy areas, Inter Tidal Zones, Island and other land parts of estuarine area were also interpreted visually. Google Earth mobile App and Avenga Map Mobile App were used in the field for locating the ground truth areas and to correlate and verify the ground information with satellite images. For further accuracy Geo Tagged photos were used to correlate the map data with ground data. These images were taken at the time of field ground truth which helped to reveal the true interpretation and investigation on land use land cover features in ground. A proper planning with the help of map (Fig. 2) was prepared for the field investigation. Centered at Gupti, the field investigation started with considering the vital and essential parameters required for all categories of land use classification. The places were taken East, West, North and South of Gupti and the places as identified for the cross checking with the satellite image and topographical sheet are Habelikhati in East, Khola in West, Dangamal in the North and Pentha in the South.

The interpreted layers were stored in GIS platform and all these items were also separated through non-spatial value like land use category. Through this process land use and land cover maps of four different years have been created using ArcGIS 10.7 software. The Unsupervised Classification Maps and False Colour Composite maps were verified by ground truth verification of land use and vegetation types. In a broader sense the interpretation of satellite images was done for 9 major classes like (a) agriculture (b) dense mangroves (c) water (waterbody of all types which includes rivers and creeks within the PA) (d) open mangroves (e) inter tidal zone (f) mudflats (g) plantations/vegetation (including village and near plantations other than mangroves) (h) sand and (i) aquaculture. Finally the statistical analysis was carried out for each year to figure out the change matrix, percentage change, individual graphs and pie charts of these referenced years.

LAND USE LAND COVER CHANGE ANALYSIS

There is change in forest cover with addition of newly formed forests in Odisha. Mangrove cover in Kandrapara district also witnessed an increase in the Mangrove cover as compared to 2017 (FSI, 2019). A detailed study of land use and land cover (LULC) of Bhitarkanika Wildlife Sanctuary for nine categories was carried out for a period of 30 years details of which are presented in Table 2.

Table 2. Change in area (km^2) of land use and land cover classes of Bhitarkanika Wildlife Sanctuary from 1990 to 2020

SL NO	Category	1990		2000		2010		2020	
		Area (km^2)	Area in %	Area (km^2)	Area in %	Area (km^2)	Area in %	Area (km^2)	Area in %
1	Agriculture	338.2	50.3	350.0	52.0	317.8	47.3	311.4	46.3
2	Dense Mangroves	118.2	17.6	122.0	18.1	122.9	18.3	101.4	15.1
3	Waterbody	82.1	12.2	80.0	11.9	80.1	11.9	75.0	11.2
4	Open Mangroves	37.2	5.5	29.8	4.4	42.0	6.2	49.5	7.4
5	Inter tidal zone	13.6	2.0	16.2	2.4	18.8	2.8	22.9	3.4
6	Mud Flats/ Swamps	47.9	7.1	33.9	5.0	42.4	6.3	48.7	7.2
7	Plantation/ Other vegetation	27.8	4.1	34.3	5.1	36.4	5.4	36.9	5.5
8	Sand	6.6	1.0	5.9	0.9	9.5	1.4	6.8	1.0
9	Aquaculture	0.4	0.1	0.6	0.1	2.4	0.4	19.5	2.9

The changes within the time period of three decades were analyzed for classes like (a) dense mangroves (b) open mangroves (c) agriculture (d) plantations / vegetation (including village and near plantations other than mangroves) (e) water (water body of all types which includes rivers and creeks within the pa) (f) mudflats (g) sand (h) inter tidal zone and (i) aquaculture (Table 3).

Table 3. Change matrix of different land use and land cover classes in Bhitarkanika Wildlife Sanctuary

Sl. No.	Category	1990	2020	Change matrix
1	Agriculture	338.2	311.36	-26.8
2	Dense mangroves	118.2	101.41	-16.8
3	Waterbody	82.1	75.01	-7.1
4	Open mangroves	37.2	49.49	12.3
5	Inter tidal zone	13.6	22.94	9.4
6	Mud flats/ swamps	47.9	48.72	0.8
7	Plantation/ Other vegetation	27.8	36.90	9.1
8	Sand	6.6	6.83	0.2
9	Aquaculture	0.4	19.45	19.0

The final land use and land cover maps were prepared after prior interpretation of individual Land sat satellite imagery of 1990, 2000, 2010 and 2020 (Fig. 3).

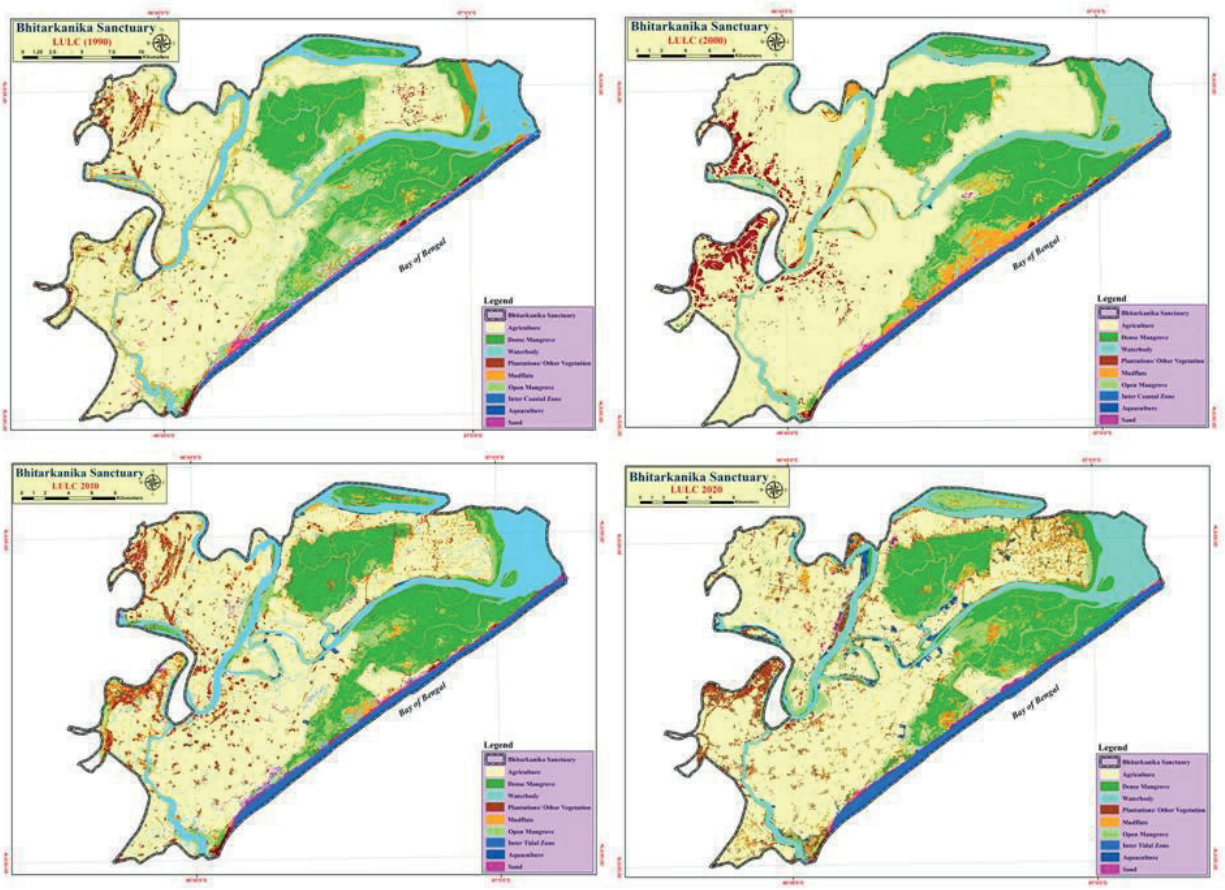


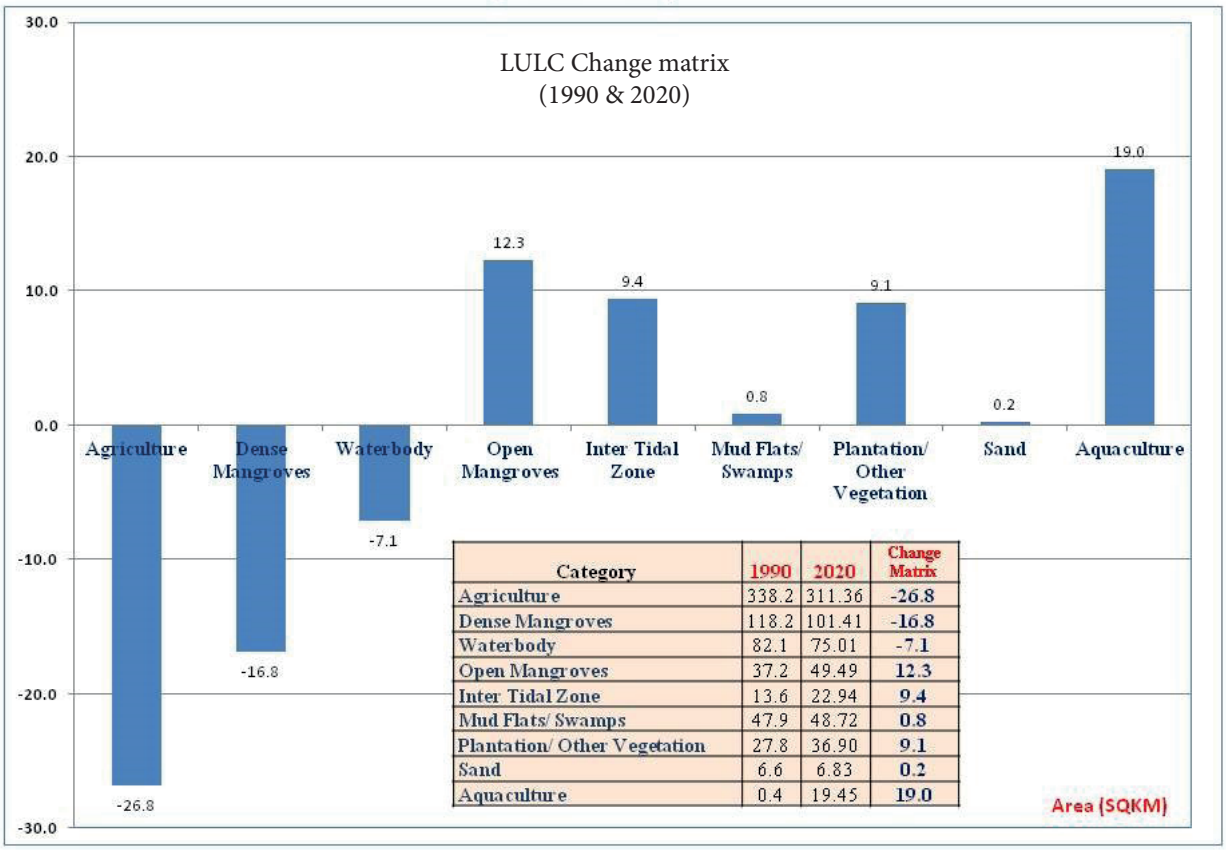
Fig. 3. Land use/cover change of different categories during years 1990, 2000, 2010 and 2020 of Bhitarkanika Wildlife Sanctuary

The analysis further continued with the study of net change area for each decade and finally the overall change in area was calculated from 1990 - 2020 (Table 4).

Table 4. Land use changes in each period in Bhitarkanika Wildlife Sanctuary (km^2)

Sl. No.	Category	Net change in 1990–2000	Net change in 2000–2010	Net change in 2010-2020	Over all Change 1990–2020
1	Agriculture	11.8	-32.2	-6.4	-26.8
2	Dense mangroves	3.8	0.9	-21.5	-16.8
3	Waterbody	-2.1	0.2	-5.1	-7.1
4	Open mangroves	-7.4	12.2	7.5	12.3
5	Inter tidal zone	2.6	2.6	4.1	9.4
6	Mud flats/ swamps	-14.0	8.5	6.3	0.8
7	Plantation/ other vegetation	6.5	2.0	0.5	9.1
8	Sand	-0.7	3.5	-2.6	0.2
9	Aquaculture	0.2	1.8	17.1	19.0

Basing on the study area analysis, considering all the four years with 3 decades time period, a change matrix was prepared (Fig. 4).



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Plantation/ Other Vegetation	27.8	36.90	9.1
Sand	6.6	6.83	0.2
Aquaculture	0.4	19.45	19.0

Fig. 4. An overview of change matrix (1990–2020) in Bhitarkanika Wildlife Sanctuary

Land use of agriculture

It is a fact from above figures that Agriculture is the predominated Land use category of all and is practiced in a major portion of the study area. But this study analysis shows that a land transformation from agricultural to aquaculture in recent years. As it was observed that during 1990, 338.2 km^2 area with 50.3% of the total area was under agriculture was increased to 350.0 km^2 with 52% in 2000. This change added some more areas under Agriculture where rice/ paddy being the major in agricultural crops. This trend declined to 317.8 km^2 in 2010 with 47.3 per cent to further to 311.4 km^2 having 46.3 per cent in 2020. The rate of decline as revealed in the study has a significant impact on Agriculture as a result of introduction of aquaculture farms within the sanctuary area. The aquaculture firms require saline water which are transformed to the aquaculture ponds through inlet channels. Actually these in turn are the carrier of irrigation water for the agricultural fields and add salinity to the fertile soil of the region which indicates possibilities of decrease in rice yield with the increasing salinity in the area. Such an enhanced salinity of the soil is expected to impart adverse effects on local economy. Several other studies on exploration of possibilities in yield reduction due to saline water intrusion have also confirmed that salinisation and subsequent acidification of agricultural soil renders considerable reduction in rice yield (White et al., 1996). Even with moderately saline water irrigation, the yield has been found affected (Asch et al., 2000). In addition, as it has been reported earlier that, a shift in highest rainfall months from June and October during 1980-84 and August to October during 1995-99, is noticed (Mitra and Hazra, 2003). Since 1988-89 the monsoon rainfall excluding the super cyclone year has been found to decline rapidly. Among other factors, climate plays most vital role in agricultural productivity. Alike other coastal zones, Bhitarkanika too, cannot depend on the irrigation water for agricultural practices, as the estuarine water may make the soil saline, if irrigated. Timely and the desired level of rainfall thus are very critical for the region.

Land use of dense mangroves

In particular to the Bhitarkanika Wildlife Sanctuary, the mangrove plays a major role in land use classification. The wetland supports one of the largest mangrove ecosystems after Sundarbans, Gujarat and Andhra Pradesh in the Indian mainland. It has more than 300 numbers of plant species, which include mangroves, mangrove associates and non mangroves. The floral diversity of Bhitarkanika wetland is known to be largest and the most productive ecosystems in India. It adds to the coastal fishery production. The rivers and creeks in the wetland are a major source of variety of indigenous fish. In addition, the local people depend on the mangrove vegetation for collection of honey, wax and medicinal plants as these dense mangroves provide valuable services to them.

The land use study in Bhitarkanika Wildlife Sanctuary shows a declining trend in Dense Mangroves since the early 90s. In 1990 only 118.2 km^2 area comprising 17.6% of the total area of the sanctuary was under Dense Mangroves category, whereas the trend continued to decline to 122.0 km^2 in 2000, 122.9 km^2 in 2010 and 101.4 km^2 in 2020 respectively with 18.1% in 2000, 18.3% 2010 and 15.1% in 2020. This study reveals the reduction in the area of dense mangroves caused by endogenic and exogenic forces which are act as an alternative landuse elements like aquaculture, agriculture and tourism which possess high level of threat to this mangrove class.

Land use of open mangroves

This is mostly found along the fringes of defunct creeks, in small islands and forest peripheries where the vegetation density is less than 40% canopy cover. The trend in change in the open mangrove cover increase, being 37.2 km^2 in 1990 with 5.5%, 29.8 km^2 in 2000 with 4.4% , 42.0 km^2 with 6.2 km^2 in 2010 and 2020 the figures rose up to 49.5 km^2 with 7.4% increase in total. This kind of vegetation is found as large patches inside the Satabhaya coast, Suneirupei forests, Gopalpur P.F. and Bhitarkanika R.F.

Land use of water body

The sanctuary is bounded by Dhamara river in the north, the Hansua in the west and the Bay of Bengal in the east and south. The river Dhamara (the confluence of Brahmani and Baitarani) in combination with Maipura, the tributary of Brahmani, constitutes the delta developed by creeks and channels in Odisha coast. The water body category includes all the rivers, creeks, channals, ponds etc. now the land use study shows decline of area under water body.This can be a change drawn between the high tides and low tides. Some portions of this water get in to the feeding channel which supply water to the aquaculture ponds. The trend chart of water body shows that 82.1 km^2 having 12.2% of area in 1990 was under this category, whereas in 2020 the land use under water body category decreased and went down to 75.0 km^2 with 11.2% decrease in area under water body. The overall change in waterbody category is found to be 7.1 km^2 less as compared to the area under water body in 1990.

Land use of inter tidal zone

The Inter Tidal Zone is the area between the sea and the shore line. As it is clearly indicative that the inter tidal zone is increasing in due course of time. Every decade some more area is added to the inter tidal zone. The direct agents acting in this scenario is the sea current and the tidal effect of the Bay of Bengal. The area under tidal zone in 1990 was 13.6 km^2 with 2% of the total area and increased on to 16.2 km^2 in 2000 with 2.4%. The increase further continued with 18.8 km^2 totaling to 2.8% of area in 2010, finally contributed 22.9 km^2 with 3.4% area under this land use category. The change in the inter tidal zone as reflected in the analysis shows an increase net change area with 2.6 km^2 in 1990–2000, similarly same area was under this category of land use in the next decade i.e. 2.6 km^2 in 2000–2010. The area increased to 4.1 km^2 in 2010-2020 making an overall net change of 9.4 km^2 from 1990 to 2020.

Land use of mud flats/ swamps

The mudflats extensive and wide salty marshy land main consists of clay and silt. Presently the mudflats contribute to 48.7 km^2 of area. They are usually submerged in the saline waters due to the effect of high tides and low tides. These mudflats usually are formed due to the deposition of sediments from river outflow from the Bhitarkanika drainage catchment area and are found in the Maipura and Dhamra estuaries which act as major inlet river in the sanctuary. The mudflats had only 47.9 km^2 contributing to 7.1% of the total area in 1990. But it the change in land use was major in the year 2000 with 33.9 km^2 and 5% area with a decrease of 14.0 km^2 area as compared to 1990. The sanctuary again had an increase of area in mudflat category figuring to 42.4 km^2 with 6.3% in 2010, it added an area of 8.5 km^2 to the net change as compared to the year 2000. Finally the mudflats contributed 0.8 km^2 area as an overall change in respect to 1990 compare with 2020.

Land use of plantation/ other vegetation

Overall plantation in the sanctuary area was taken in account for 3 decades. This category included all the vegetation and plantations other than mangrove species. This category of the land use witnessed an overall increase in area with increasing trend in all decades. With 27.8 km^2 area having 4.1 per cent area under this category in 1990, it started an onward trend in subsequent years. It noticed a change in area in 2000 with 5.1 per cent having 34.3 km^2 area with an increase of 6.5 km^2 area as compared to 1990. In the year 2010 plantation category increased to 36.4 km^2 with 5.4 per cent and finally reached to 36.9 km^2 having 5.5 per cent area under it. The overall change in area as compared to 1990 with 2020, 9.1 km^2 of area contributed to this category. There is a marginal change in area which is due to other plantation activities done by the village people and the roadside plantation/ conservation activity of the government.

Land use of sand

This land use category is confined to the coastal areas along the Gahirmatha Marine Sanctuary coast adjacent to the inter tidal zone.

The area along the Gahirmatha coast is found suitable for the Olive Ridley sea turtles which come for mass nesting and egg laying process every year. Along this coast some Casuarina plantation was observed near Habelikhatti, Sathbhaya, Kanhupur and Barunai. The sand category had a marginal influence in area as compared to all 3 decades. It had only 6.6 km^2 area with 1.0 per cent in 1990 and in 2020 it was observed with an area of 6.8 km^2 having 1.0 per cent under this landuse category. It observed only 0.2 km^2 increase in area from 1990 to 2020 in its overall change area.

Land use of aquaculture

In and around the site, a large chunk of the agriculture land adjacent to rivers and creeks have been converted to prawn farms. The existing population pressure and mass unemployment has invited a large number of people residing nearby and from outside the area have purchased private land along the coast as well as along the rivers and the creeks, converting the same to aquaculture farms. They are discharging the untreated effluents from the farm to nearby rivers and creeks and thereby affecting the aquatic fauna and the mangroves. As these farms are in close proximity to the mangroves of these region they impact factor is very high in degrading the mangroves. The rate in increase of aquacultural farms has a direct impact on the declining rate on agricultural lands and associated activities. Moreover the inlet to these ponds which acts as feeder channels are usually rivers and creeks passes through mangroves and agricultural fields. Due to this salinity of the area increases which results in decrease in rice and paddy yield. As it is clearly confirmed from the study that in 1990 only 0.4 km^2 with 0.1 per cent area was under aquaculture and the nature was scattered type. The rate of increase was marginal in the two decades up to 2010 as the area under this category was 0.6 km^2 in 2000 and 2.4 km^2 in 2010 with 0.6 per cent and 0.4 per cent of the total area. Gradually the rate and the area under aquaculture started to increase, in 2020 the aquaculture farms/ponds had steep increase with 19.5 km^2 areas totaling to 2.9 per cent. This sudden spike in aquaculture was because it is a very profitable business as the local price of aquacultural products is quite high in comparison to other fishery items. The rapid commercialization of this business has added to the existing precarious situation of mangroves in the study area. The aquacultural farms directly affect the process of degradation of mangroves in the area by blocking or diverting the stream channels towards their farms.

RESULTS AND DISCUSSION

Mangrove forests in the Bhitarkanika Wildlife Sanctuary are getting degraded due to intense anthropogenic activities (Misra et al., 2018). People do have associated activities on the mangroves and extract them for economic and medicinal purposes. The increase of aquaculture ponds and the daily discharge of effluents from these ponds to the rivers and creeks have got severe impact on degradation of dense mangroves. The daily need and dependency on mangrove forests by these people are depleting the density of mangroves which are disappearing at an alarming rate. Many of the mangroves or associated species which have their economic importance have become threatened because of there over use and exploitation due to various human activities. It is observed that, government has taken some initiatives for the sustainable development in the study area. Some degraded areas are now under plantation. The settlement distribution is rapidly increased which is adding tremendous pressure in the dynamic coastal environment. The government and the forest department should take adequate steps for realising all these pressures on mangroves by ensuring different *in-situ* and *ex-situ* conservation activities. The managers of the sanctuary and mangrove areas should come up with some alternative ideas and projects which will help the local people dependent on mangroves and associated species that makes their livelihood different to reduce such pressures on the mangroves.

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