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Advances in Life Sciences

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MINI REVIEW

Microbial Fuel Cell: A New Source of Green Energy

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ABSTRACT

A microbial fuel cell is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms. Microbial fuel cell technology represents a new form of renewable energy by generating electricity from what would otherwise be considered waste, such as industrial wastes or waste water etc. Microbial Fuel Cells have the potential to simultaneously treat wastewater for reuse and to generate electricity; thereby producing two increasingly scarce resources. It involves biological reactions converting chemical energy present in the bonds of organic compounds into electric energy, through the reactions of microorganism in aerobic conditions. Unlike other traditional methods of power generation it does not produce any toxic gases or chemicals into the environment. Biological treatment becomes more feasible and simpler with the use of MFC's. Microbial fuel cells have a number of potential uses. The most readily apparent is harvesting electricity produced for use as a power source. Virtually any organic material could be used to feed the fuel cell, including coupling cells to wastewater treatment plants. Sludge production in the waste water treatment is expected to be less under MFC treatment when compared to other conventional methods, as the major part of energy stored in organic wastes is converted to electricity, and the remaining energy is utilized for microbial growth. The main economic gain from treating wastewater with MFCs will be biological oxygen demand (BOD) removal efficiency without non-renewable energy consumption rather than municipal electric power generation.

Key words: *Microbial fuel cell, electrical energy, biological reaction, organic waste.*

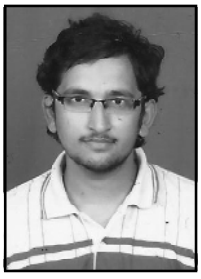
The use of fossil fuels, especially oil and gas, for all human needs in recent years has accelerated and this triggers the global energy crisis. Renewable bioenergy is viewed as one of the ways to decrease the current global warming crisis. It is well known that fuels, such as ethanol, butanol, methane and hydrogen can produce by microorganisms. But the electricity production using microbes, which is known as microbial fuel cells (MFCs), is recent development in energy biology and highly attracting area. Microbial fuel cells put forward the possibility of harvesting electricity from organic waste and renewable biomass. These are attractive sources of energy because they are 'carbon-neutral'.

What is microbial fuel cell?

Microbial fuel cells are devices which convert organic matter to energy using microorganisms as catalysts. Generally bacteria are used in MFCs to generate electricity while accomplishing the biodegradation of organic matters or wastes. Figure 1 shows a schematic diagram of a typical MFC for producing electricity. It consists of anodic and cathodic chambers partitioned by a proton exchange membrane (PEM). The anode compartment is typically maintained under anoxic conditions, whereas the cathode can be suspended in aerobic solutions or exposed to air. Electrons flow from the anode to the cathode through an external electrical connection that typically includes a resistor, a battery to be charged or some other electrical device.

Microbes in the anodic chamber of an MFC oxidize added substrates and generate electrons and protons in the process. Carbon dioxide is produced as an oxidation product. Unlike in a direct combustion process, the electrons are absorbed by the anode and are transported to the cathode through an external circuit. After crossing a PEM or a salt bridge, the protons enter the cathodic chamber where they combine with oxygen to form water. Microbes in the anodic chamber extract electrons and protons in the dissimilative process of oxidizing organic substrates.

Mani Vinay Kumar Kotipalli is pursuing studies at National Insititute of Technology Warrangle, Andhra Pradesh. He worked as a Research Assistant at the Laboratory of Molecular and Cellular Medicine, University of British Columbia, Canada. During this period, he gained experience with Immunohistochemistyr and High Resolution Imaging. He also worked on 'Characterization of isolets of Insulin gene knockout mice' as a part of the Diabetes Research Group of Prof. Timothy J. Kieffer. At present he is working on regeneration of bone material by using Mesenchymal Stem cells.



Rakesh Santhapur has research experience on Microbial Fuel Cell. Presently he is working on screening of cellulose producing microbes and optimizing cellulose production. He is persuing his studies at National Institute of Technology, Warangle, Andhra Pradesh.

Construction and Design of MFC

There are basic components of MFCs which are important in constructions. Electrodes, wirings, glass cell and salt bridge have an important role. Salt bridge is replaced with Proton exchange membrane in PEM fuel cell. Though it enhances the cost but handling and the power generation both get enhanced, thus increasing the portability and efficiency of the system. Anodic material must be conductive, bio compatible, and chemically stable with substrate. Metal anodes consisting of noncorrosive stainless steel mesh can be utilized, but copper is not useful due to the toxicity of even trace copper ions to bacteria. The simplest materials for anode electrodes are graphite plates or rods as they are relatively inexpensive, easy to handle, and have a defined surface area. Much larger surface areas are achieved with graphite felt electrodes. The most versatile electrode material is carbon, available as compact graphite plates, rods, or granules, as fibrous material (felt, cloth, paper, fibers, foam), and as glassy carbon. Proton Exchange Membrane is usually made up of NAFION or ULTREX. Apart from that fuel cells can be classified in two types on the basis of number of compartments or chambers.

Double chambered fuel cells

Both the cathode and anode are housed in different compartments or chambers connected via a proton exchange membrane (PEM) or sometimes salt bridge. PEM or salt bridge mainly functions as medium for transfer of proton to make the circuit complete as shown in figure 1A. This not only completes the reaction process but also prevents anode to come in direct contact with oxygen or any other oxidizers. They are run in batches and can be used for producing higher power output and can be utilized to give power in much inaccessible conditions. It can be suitable designed to scale up to treat large volume of wastewater and other source of carbon. These particular types are called up flow mode of microbial fuel cell as shown figure 1 B. They practically fall between the classification of single chambered and double chambered microbial fuel cells. They are mediator-less and sometimes membrane-less and can be used for large scale production of electricity from the wastes.

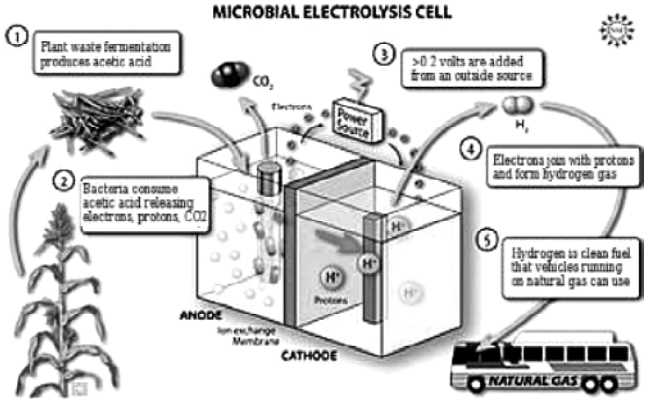


Fig. 1. Microbial Fuel Cell- General Overview.

Single Chambered Fuel cells

They are simple anode compartment where there is no definitive cathode compartment and may not contain proton exchange membranes as shown in Figure 2. Porous cathodes form one side of the wall of the cathode chamber utilizing oxygen from atmosphere and letting protons diffuse through them. They are quite simple to scale up than the double chambered fuel cells and thus have found extensive utilization and research interests lately. The anodes are normal carbon electrodes but the cathodes are either porous carbon electrodes or PEM bonded with flexible carbon cloth electrodes. Cathodes are often covered with graphite in which electrolytes are poured in steady fashion which behaves as catholytes and prevent the membrane and cathode from drying. Thus water management or better fluid management is an important issue in such single chambered fuel cells.

Stacked Microbial fuel cells

These are another type of construction in which fuel cells are stacked to form battery of fuel cell.[18] This type of construction doesn't affect each cell's individual Columbic efficiency but in together it increases the output of overall battery to be comparable to normal power sources as shown in figure 3. These can be either stacked in series or stacked in parallel. Both have their own importance and are high in power efficiency and can be practically utilized as power source.

Working of Microbial Fuel Cells

The basic working principle of MFC is same as that of the electrolytic cell. Oxygen is most suitable electron acceptor for an microbial fuel cell due to its high oxidation potential, availability, sustainability and lack of chemical waste product, as the only end product is water.

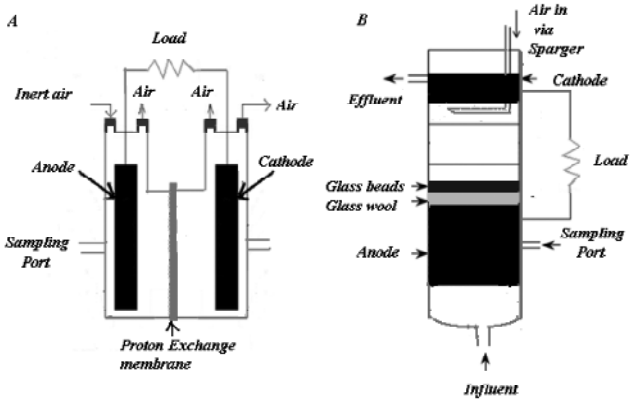


Fig. 2. A) Simple design of Double chambered Microbial Fuel Cell B) Schematic Designs of Cylindrical Membrane-less fuel Cells.

Typical electrode reactions are shown below using acetate as an example substrate.

Anodic reaction:

$$CH_3COO^- + 2H_2O \xrightarrow{\text{Microbes}} 2CO_2 + 7H^+ + 8e^-$$

Cathodic reaction: $O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O$

The overall reaction is the breakdown of the substrate to carbon dioxide and water with a concomitant production of electricity as a by-product. Based on the electrode reaction pair above, an MFC bioreactor can generate electricity from the electron flow from the anode to cathode in the external circuit.

Electrons produced by bacteria from these substrates are transferred to anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material. Protons move to cathodic compartment through Proton Exchange Membrane and complete the circuit. Microbial fuel cells use inorganic mediators to tap into the electron transport chain of cells and steal the electrons that are produced. The mediator crosses the outer cell lipid membranes and plasma wall; it then begins to liberate electrons from the electron transport chain that would normally be taken up by oxygen or other intermediates. The now-reduced mediator exits the cell laden with electrons that it shuttles to an electrode where it deposits them; this electrode becomes the electro-generic anode (negatively charged electrode). The release of the electrons means that the mediator returns to its original oxidized state ready to repeat the process. It is important to note that this can only happen under anaerobic conditions, if oxygen is present then it will collect all the electrons as it has a greater electronegativity than the mediator. Organic substrates are utilized by microbes as their energies are transferred to electron acceptor (molecular oxygen) in absence of such electron acceptors micro-organisms shuttle electron into anode surface

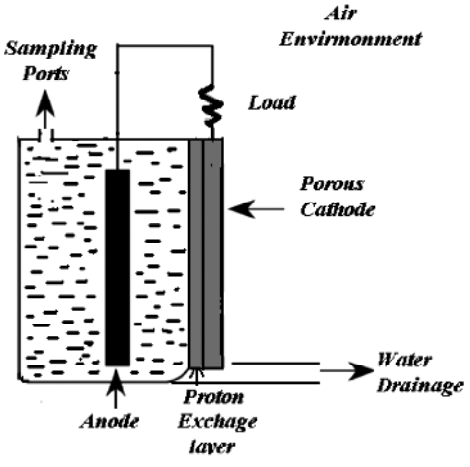


Fig. 3. Schematic design of Single chambered Microbial Fuel Cell.

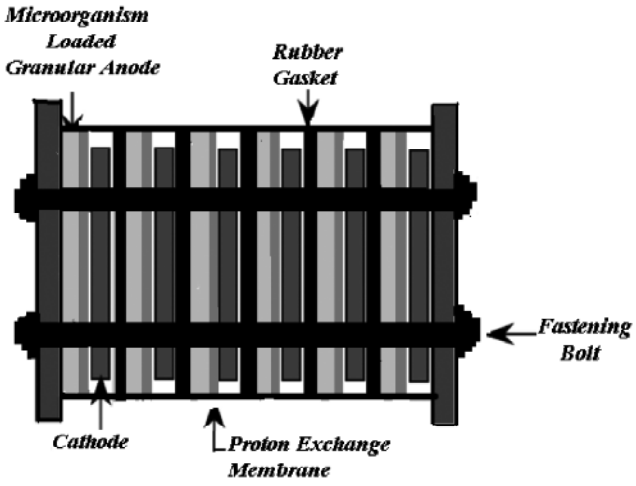


Fig. 4. Schematic Design of Stacked type Microbial Fuel Cell.

with help of mediators. However few micro-organisms are able to transfer electrons directly to electrode. This type of system is called as Mediator Less Microbial Fuel Cell. Examples of such micro-organisms which are currently available are: Shewanella oneidensis, Geobacter metallireducens etc. Mediator Less Microbial Fuel Cell has more commercial potential as mediators are expensive and sometimes toxic to microorganisms.

Mechanisms for electron transport to electrodes

In microbial fuel cells, the electrons liberated from the organic matter are transferred to electrodes and generates the electricity. So this is the key mechanism one has to understand for the efficient conversion of waste to electricity generation. There are three primary mechanisms are proposed for microorganisms to transfer electrons to electrodes.

Electron transport by artificial mediators

In this proposed mechanism electrons are transported

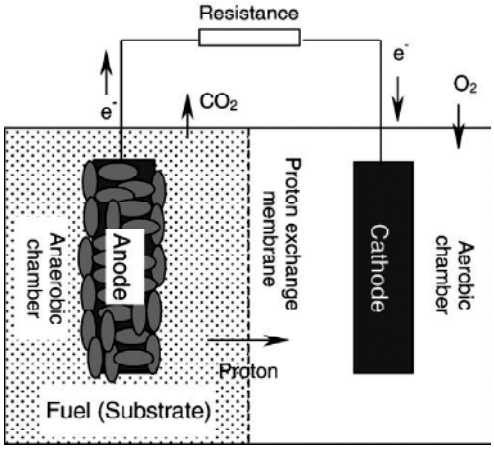


Fig. 5. Schematic diagram of typical two-chamber microbial fuel cell

by artificial mediators, sometimes referred to as electron shuttles. This chemical materials offer the possibility for microorganisms to generate reduced products that are more electrochemically active than most fermentation products. These electron shuttles are typically capable of crossing cell membranes, accepting electrons from one or more electron carriers within the cell, exiting the cell in the reduced form and then transferring electrons onto the electrode surface. Mediators are important in microbial fuel cells which use microorganisms such as Escherichia coli, Pseudomonas, Proteus, and Bacillus species that are unable to effectively transfer electrons derived from central metabolism to the outside of the cell. The Commonly used electron shuttles include, thionine, benzylviologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1, 4-naphthoquinone and various phenazines, phenothiazines, phenoxoazines, iron chelates and neutral red. The mediators should posses the following characters for efficient electron transportation (1) able to cross the cell membrane easily; (2) able to grab electrons from the electron carries of the electron transport chains; (3) possessing a high electrode reaction rate; (4) having a good solubility in the anolyte; (5) non-biodegradable and non-toxic to microbes; (6) low cost.

Electron transport through microorganism's own mediator

It is also known that some microorganisms can produce their own mediators to promote extracellular electron transfer. This was first proposed as a mechanism to facilitate electron transfer to Fe^{3+} in Shewanella oneidensis. Other organisms, such as Geothrix ferementans and Pseudomonas species also produce electron shuttles. Biosynthesizing an electron shuttle is energetically expensive and therefore an electron shuttle

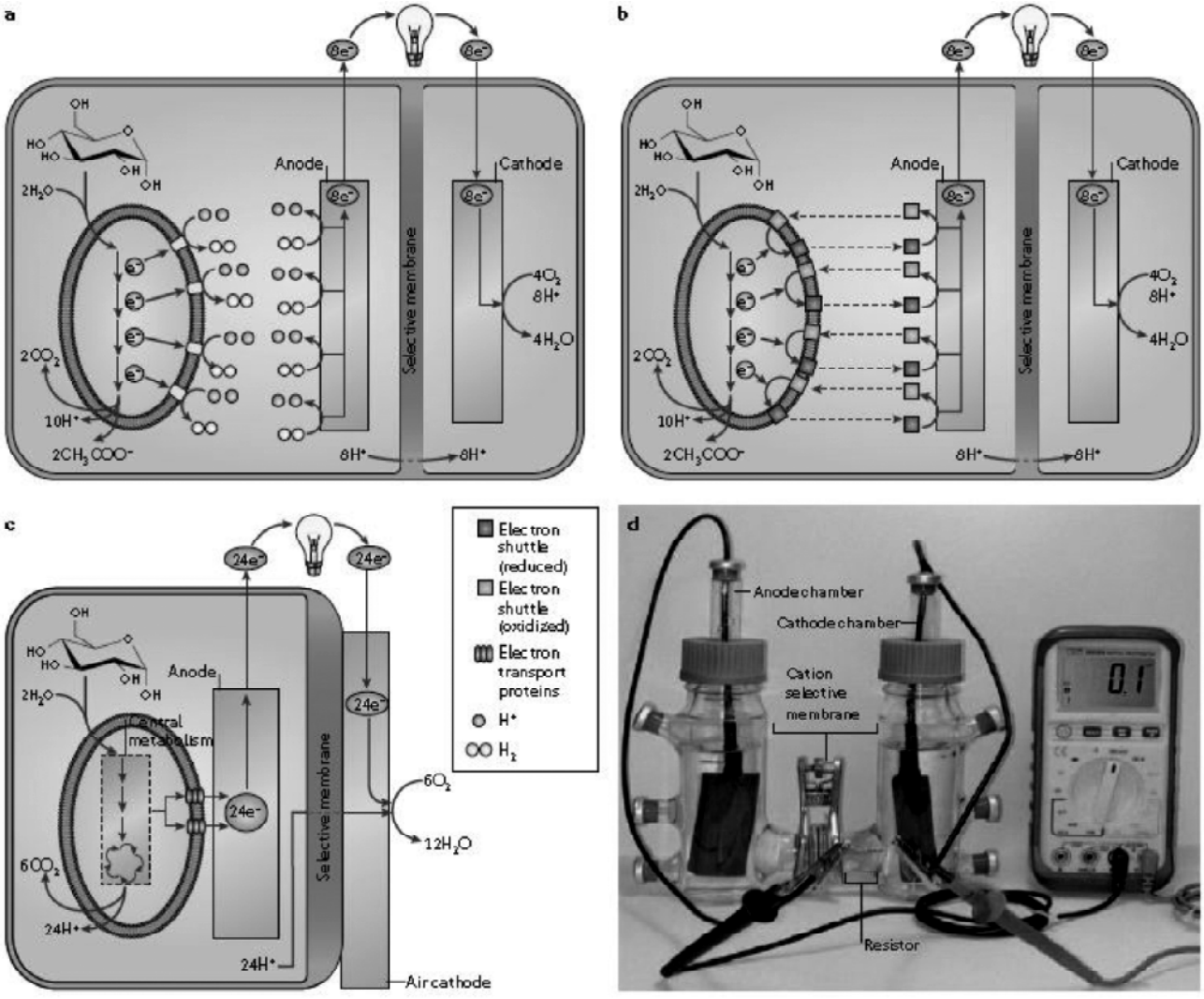


Fig. 6. Examples of microbial fuel cells producing electricity through different mechanisms of electron transfer to the anode. A. An indirect microbial fuel cell. B. A mediator-driven microbial fuel cell. C. The oxidation of glucose to carbon dioxide with direct electron transfer to the electrode surface. D. A two-chambered microbial fuel cell.

must be recycled many times in order to recoup this energy investment. For this reason, microorganisms that produce electron shuttles are expected to be at a competitive disadvantage in open environments in which the shuttle will rapidly be lost from the site of release. Significant limiting factor in electricity production by several microorganisms that produce an electron shuttle is that they only incompletely oxidize their organic fuels.

Direct electron transfer

It was first proposed that microorganisms might be able to transfer electrons to an electrode surface when it was discovered that cultures of Shewanella putrefaciens produced electricity while metabolizing lactate. However, this was prior to the discovery, discussed above, that Shewanella species produce an electron shuttle, which could account for the electron transfer to the electrode. It was proposed that electrons might be directly transferred from the cell to the electrode through outer-membrane c-type cytochromes48, but no direct evidence for this was provided. Furthermore, it is now recognized that outer-membrane cytochromes are important in electron shuttle reduction in Shewanella.

Microbes used in Microbial Fuel Cells

Many microorganisms possess the ability to transfer the electrons derived from the metabolism of organic matters to the anode. A list of them is shown in Table (1) together with their substrates. Marine sediment, soil, wastewater, fresh water sediment and activated sludge are all rich sources for these microorganisms. A number of recent publications discussed the screening and identification of microbes and the construction of a chromosome library for microorganisms that are able to generate electricity from degrading organic matters.

Factors affecting the MFCs efficiency

Electrode Material

Type of material used in electrode preparation will show vital effect on MFCs efficiency. Better performing electrode materials usage will always improve the performance of MFC because different anode materials result in different activation polarization losses. Pt and Pt black electrodes are superior to graphite, graphite felt and carbon-cloth electrodes for both anode and cathode constructions, but their costs are much higher. MFCs with Pt or Pt-coated cathodes yielded higher power densities than those with graphite or graphite felt cathodes.

pH Buffer and Electrolyte

If no buffer solution is used in a working MFC, there will be an obvious pH difference between the anodic and cathodic chambers, though theoretically there will be no pH shift when the reaction rate of protons, electrons and oxygen at the cathode equals the production rate of protons at the anode. The PEM causes transport barrier to the cross membrane diffusion of the protons, and proton transport through the membrane is slower than its production rate in the anode and its consumption rate in the cathode chambers at initial stage of MFC operation thus brings a pH difference. However, the pH difference increases the driving force of the proton diffusion from the anode to the cathode chamber and finally a dynamic equilibrium forms. Some protons generated with the biodegradation of the organic substrate transferred to the cathodic chamber are able to react with the dissolved oxygen while some protons are accumulated in the anodic chamber when they do not transfer across the PEM or salt bridge quickly enough to the cathodic chamber. It was possible that the buffer compensated the slow proton transport rate and improved the proton availability for the cathodic reaction. This again suggests that the proton availability to the cathode is a limiting factor in electricity generation. Increasing ionic strength by adding NaCl to MFCs also improved the power output possibly due to the fact that NaCl enhanced the conductivity of both by anolyte and the catholyte.

Proton Exchange System

Proton exchange system can affect an MFC system's internal resistance and concentration polarization loss and they in turn influence the power output of the MFC. Nafion (DuPont, Wilmington, Delaware) is most popular because of its highly selective permeability of protons. However, side effect of other cations transport is unavoidable during the MFC operation with Nafion. But its usage is better in the sense of charge balance between the anodic and cathodic chambers. Hence Nafion as well as other PEMs used in the MFCs are not a necessarily proton specific membranes but actually cation specific membranes. The ratio of PEM surface area to system volume is important for the power output. The MFC internal resistance decreases with the increase of PEM surface area over a relatively large range. Membranes are prone to fouling if the fuel is something like municipal wastewater. Membrane-less MFCs are desired if fouling or cost of the membrane becomes a problem in such applications.

Operating conditions in the anodic chamber

Substrate type, concentration and feed rate are important factors that impact the performance of an MFC. Power density varies greatly with different substrates using

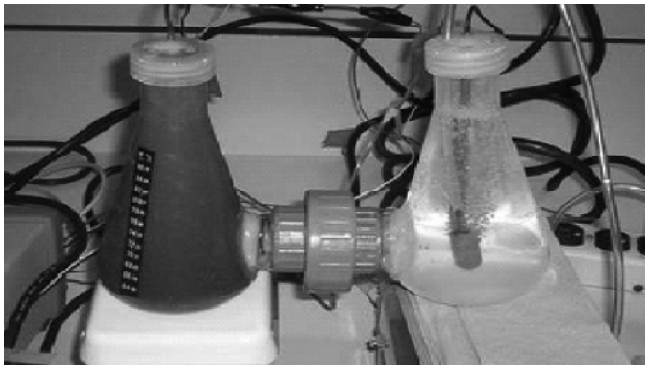


Fig. 7. A real time Microbial Fuel Cell

Table 1. Microbes used in microbial fuel cells

Microbes	Substrate	Applications
Actinobacillus succinogenes	Glucose	Neutral red or thionin as electron mediator
Aeromonas hydrophila	Acetate	Mediator-less MFC
Alcaligenes faecalis, Enterococcus	Glucose	Self-mediate consortia isolated from MFC with a maximal level of 4.31 W m? 2.
Gallinarum, Pseudomonas aeruginosa	Starch, glucose	Fermentative bacterium
Clostridium beijerinckii	Starch, glucose, lactate, molasses	Fermentative bacterium
Clostridium butyricum	Starch, glucose, lactate, molasses	Sulphate/sulphide as mediator
Desulfovibrio desulfuricans	Sucrose	Ferric chelate complex as mediators
Erwinia dissolven	Glucose	Ferric chelate complex as mediators
Escherichia coli	Glucose sucrose	Mediators such as methylene blue needed.
Geobacter metallireducens	Acetate	Mediator-less MFC
Geobacter sulfurreducens	Acetate	Mediator-less MFC
Gluconobacter oxydans	Glucose	Mediator (HNQ, resazurin or thionine) needed
Klebsiella pneumoniae	Glucose	HNQ as mediator biomineralized manganese as electron acceptor
Lactobacillus plantarum	Glucose	Ferric chelate complex as mediators
Proteus mirabilis	Glucose	Thionin as mediator
Pseudomonas aeruginosa	Glucose	Pyocyanin and phenazine-1-carboxamide as mediator
Rhodoferax ferrireducens	Glucose, xylose, sucrose, altose	Mediator-less MFC
Shewanella oneidensis	Lactate	Anthraquinone-2,6-disulfonate (AQDS) as mediator
Shewanella putrefaciens	Lactate, pyruvate, acetate, glucose	Mediator-less MFC but incorporating an electron mediator like Mn(IV) or NR into the anode enhanced the electricity production
Streptococcus lactis	Glucose	Ferric chelate complex as mediators

same a given microbe or microbial consortium. Electricity generation is dependent on substrate concentration both in batch and continuous-flow mode MFCs. Usually a higher substrate concentration yields a higher power output in a wide concentration range. Interestingly, the electricity generation in an MFC is often higher at a relatively low level of feed rate before heading downward. This may be because a high feed rate promoted the growth of fermentative bacteria faster than those of the electrochemically active bacteria in a mixed culture. However, if microbes are growing around the electrodes as biofilms, the increased feed rate is unlikely to affect the flora. Another possible reason is that the high feed rate brings in other alternate electron acceptors competing with the anode to lower the output.

Operating conditions in the cathodic chamber

Oxygen is the most commonly used electron acceptor in MFCs for the cathodic reaction. Power output of an MFC strongly depends on the concentration level of electron acceptors. Using hydrogen peroxide solution as the final electron acceptor in the cathodic chamber may increase power output and current density.

Surely changing operating conditions can improve the power output level of the MFCs. The bottlenecks responsible for the low power output are 1. Low rate of metabolism of the microbes in the MFCs, 2. The biotransformation rate of substrates to electrons has a fixed ceiling which is inherently slow.

Applications of MFCs

MFCs have very broad range of application including:

- Electricity generation
- Bio-hydrogen production
- Waste water treatment
- Biosensors
- Bioremediation

Future Perspectives

At present the field of MFCs is in its infancy and also this is an exciting time in microbial fuel cell research. The MFCs technology has evolve to compete with well advanced methanogenesis technology where biomass is used as substrate. In contrast to methanogenesis, MFCs are capable to convert biomass to electricity at low temperatures and substrate concentration. The discovery and usage of new anodophilic microbes that vastly enhance the electron transport rate from the biofilm covering an anode to the anode are much needed to improve the power density output in MFCs.

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MINI REVIEW

Anaemia : Past, Present and Future Scenario in India

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ABSTRACT

Anemia is a major problem seen in most of the Indian pregnant women. Specially, Iron deficiency with anemia or without anemia has adverse effects on nervous system, physical response and pregnancy outcome (around 80%). Most of the anaemias are due to inadequate supply of nutrients like iron, folic acid and vitamin B12. . Although anaemia is widespread in every Indian state, it varies considerably among states. Though the Indian Government has made lots of efforts to combat with the anaemia, still the burden of anaemia is again increasing day by day and leading to long term damage to Indian development. New and innovative strategies are needed, particularly those that improve the overall health and nutrition status. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anaemia in a given setting and population group.

Key words: *Anaemia, iron deficiency, maternal, mortality, pregnancy*

Anaemia has been universally recognized as the commonest forms of malnutrition occurring in the world. Anemia continues to be a major public health problem worldwide, particularly among females of reproductive age in developing country settings. These affect approximately 2 billion people, 80% of whom live in the developing world. However, its distribution is not uniform throughout the world. Southeast Asia has the highest levels at 79%. The Indian subcontinent alone contains nearly half the world's anaemic women (Freire, 1997). In the WHO classification of countries by the significance of anaemia as a public health issue, India is listed as having a severe problem of anaemia in both pregnant women and non-pregnant women of reproductive age (de Benoist, *et al.*, 2008).

Anemia is blood related diseases, in which the oxygen carrying capacity is been reduced due to the destruction of the hemoglobin or the RBCs level from its normal range. While many parts of the body help make red blood cells, most of the work is done in the bone marrow. Bone marrow is the soft tissue in the center of bones that helps to form blood cells. Healthy red blood cells last between 90 and 120 days. Parts of our body then remove old blood cells. Hemoglobin is the oxygen-carrying protein inside red blood cells. It gives red blood cells their red color. People with anemia do not have enough hemoglobin.

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Dr. Renu Mogra has done Ph.D. in Food & Nutrition, working as Professor for last 20 years. She is gold Medalist and has 17 years of experience in teaching, research, extension activities related to Foods & Nutrition. She has authored three practical manuals, one book; three booklets and contributed many chapters in books. She has published many research papers in the journal of national and international repute.

Causes and Consequences of anaemia: Although anaemia is multifactorial and several causes usually coexist, the most important contributor to the condition is iron-deficiency, so much so that Iron-Deficiency Anaemia (IDA) is often used synonymously with anaemia, since approximately half the cases can be attributed to iron-deficiency (Kalaivani, 2009). Most of the anaemias are due to inadequate supply of nutrients like folic acid and vitamin B12,proteins, amino acids, vitamins A, C, and other vitamins of B-complex group *i.e.*, niacin and pantothenic acid are also involved in the maintenance of haemoglobin level. Although anaemia affects people of both genders at all stages of the life cycle, it is more prominent in women of reproductive age. This is because the most dramatic

and serious health consequences of anaemia are seen in these groups and iron deficiency with anemia or without anemia has many adverse effects on nervous system, physical response and pregnancy outcome. During pregnancy, there is great demand for iron to meet of requirement for red blood cell mass expansion in mother, fetus and placental blood loss at delivery in addition to increased occult gastrointestinal blood loss. It has been well established that maternal anaemia is associated with higher risk of maternal and child mortality and poor perinatal outcomes , inadequate iron stores for the newborn, lowered physical activity, mental concentration, and productivity (Gillespie and Johnson, 1998; Ali, *et al.*, 2008 and Rohilla, *et al.,* 2010). Other health consequences include reduced immunity, increased susceptibility to heavy metal (including lead) poisoning. Iron supplementation at a later age may not reverse the effects of moderate to severe iron deficiency anemia that occurred during the first 18 months of life (Zlotkin, 2002).

Investigations: Hemoglobin estimation and study of peripheral smear is good indicator for diagnosis of anemia. There may be several methods for estimation of Hb. However in spite of limitation of present method of Hb estimation, it is a useful method of diagnosis for anemia. If the peripheral smear looks pale, there is hypochromia (large central vacuoles) and microcytosis (small deformed red cells). It suggests iron deficiency. Another test, Complete Blood Count is been done by the physician to check the anemia level; Other special laboratory investigations total iron binding capacity (TIBC), serum ferritin (SF), serum folic acid, bone marrow studies are not available everywhere and expensive. Therefore they are not for routine use to diagnose pregnancy anemia (Singla, *et al.,* 1997).

Anaemia in India – past and present: In our country more than 50 % of the total population is affected by IDA, about 25- 50 % girls became anemic by the time they reach their menarche. Anaemia is the most common complication during pregnancy in India. In a study of the Indian Council of Medical Research (ICMR Task Force Study, 1989) prevalence of anemia in pregnant rural women of 11 States reported that 87.6 per cent women had hemoglobin (Hb) <10.9 g/dl. Hemoglobin estimations in rural pregnant women in Varanasi showed 94.5, 95.3 and 95.9 per cent prevalence of anaemia in I, II and III trimesters (Agarwal, *et al.,* 2000). From time to time several studies have been done in India to demonstrate the prevalence of anaemia. The National Family Health Survey - 2 using hemocue system reported prevalence of anaemia as 49.7 per cent in pregnant women; 56.4 per cent in breastfeeding non pregnant; and 50.4 per cent among non pregnant non breastfeeding women (NFHS, 2002) and according to the last round of the National Family Health Survey, 55.3 per cent of the women were found to be anaemic across the country showing an increase from the last NFHS survey. Although anaemia is widespread in every Indian state, it varies considerably among states. The high prevalence of anaemia and malnutrition in Rajasthan – 53.8 per cent ever married and 61 per cent pregnant women suffer from anaemia has been an issue of concern (NFHS, 2006). According to the ICMR survey 1992 Andhra Pradesh had lowest percentage of anemic but the present situation has been changed considerably, amongst the four southern states, Kerala has the lowest prevalence of anaemia among its women at 32.8 per cent. In each of the other three states – Andhra Pradesh, Tamil Nadu and Karnataka, more than half the women are anaemic. In Karnataka, the number of women with anaemia went up from 42.4 per cent in NFHS-2 to 51.5 per cent in NFHS-3. About 17 per cent of Karnataka's women suffer from moderate to severe anaemia (IIPS and Macro International, 2008). The status of anemia among pregnant women and adolescent girls from various district of different states of India has been studied and gave a clear picture of higher prevalence of anaemia among adolescent and women (Choudhary, *et al.,* 2006; Bharti, *et al.,* 2009) under the anemia prevention and control program of the Government of India, iron and folic acid tablets are distributed to pregnant women, but no such program exists for adolescent girl.

National nutritional anaemia control programme in India: The programme focuses on three vital strategies: promotion of regular consumption of foods rich in iron, provisions of iron and folate supplements in the form of tablets to the high risk groups, and identification and treatment of severely anemic cases. Pregnant women are recommended to have one tablet containing 100 mg of iron and 500 mg of folic acid, per day for 100 days after the first trimester of pregnancy; a similar dose applies to lactating women and IUD acceptors. Preschool children (ages 1-5 years) are recommended to take one small tablet containing 20 mg iron and 100 mg folic acid per day for 100 days every year. Despite the measures taken to control anaemia in pregnancy and lactation in the last two decades, the severity of nutritional anaemia continues to remain a public health issue of great magnitude, suggesting that these measures have been largely ineffective. An evaluation by an ICMR task force in 11 states during 1985-86 indicated very low coverage and poor performance of the programme (ICMR Task Force Study, 1989). Interstate differences particularly in fertility, women education, nutrition status and occupation; availability of antenatal services and iron folate tablets were considered as possible factors responsible for differences in prevalence of anemia.

Future projections and conclusion: Given the multifactorial nature of this disease, correcting anaemia requires an integrated approach. In order to effectively combat it, the contributing factors must be identified and addressed. Food based approaches to increase iron intake through food fortification and dietary diversification are important, sustainable strategies for preventing IDA in the general population. In settings where iron deficiency is not the only

cause of anaemia, approaches that combine iron interventions with other measures are needed. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anaemia in a given setting and population group. Improving women's overall nutrition status and their access to resources (income) will have the greatest impact on reducing anemia in India (Creed-Kanaashiro, *et al.,* 2000). Integrated programs for hookworn eradication, malaria prophylaxis, or that address other micronutrient deficiencies would also be important for reducing the burden of anemia (Gillespie and Johnston, 1998). Making improvements in household socioeconomic status and maternal education will also affect maternal health and nutrition in a sustained way. In conclusion, the high prevalence of anemia among women in India is a burden for them, for their families, and for the economic development and productivity of the country. Iron supplementation programs, for a variety of reasons, have not been effective in reducing anemia prevalence and operational research on how best to improve existing iron supplementation programs is needed. New and innovative strategies are needed, particularly those that improve the overall health and nutrition status of adolescent girls before they enter their reproductive years (Kananni and Poojara, 2000). This will require tailored programs that target women in all socioeconomic groups and who live within both rural and urban areas, but particularly in need of intervention are the urban poor, who are a rapidly growing marginalized segment of the Indian population.

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MINI REVIEW

Accumulation of Heavy Metals in Different Water Bodies by Biological Source Algae

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ABSTRACT

Heavy metals contamination in the environment is a major global concern, Heavy metals are released into the environment from a wide range of natural and anthropogenic sources. This problem provoked the emergence of phytoremediation technologies for cleaning aquatic environment. Algae act as biological filters, bio-indicators by accumulating heavy metals like Hg, CO, Cd, Cu, Cr, Fe, Mn, Mo, Ni, Pb, Ti and Zn from the surrounding environments. This study aimed the importance of algae in accumulation of heavy metals, involved mechanism and suggested remedial measures for the preservation and restoration of the aquatic ecosystem including both fresh and marine ecosystem. The different algal species have the ability to accumulate specific heavy metals in huge amount. The algae maintain an equilibrated relation with heavy metal ions which are present in the surrounding. Algae, fungi and some eukaryotic photosynthetic organisms, produce peptides which are capable to bind heavy metals. This technology is gaining huge public acceptance due to the microbial activity and its insitu applicability which results in non-toxic end products.

Key words: *Phytoremediation, biological filters, metallothioneins, eutrophication.*

Heavy metals are natural constituents of the Earth's crust, they are stable metals or metalloids and its density is greater than 5 g/cm^3; like mercury, cadmium, cobalt, lead, molybdenum, nickel, copper, zinc etc (Nies, 1999). Heavy metals water pollution caused by industrial waste effluents is now a global problem (Jana, 1988). The heavy metals present in aquatic environment cause severe damage to aquatic life. During the biological treatment of waste water these metals kill micro-organisms delay the process of water purification. The heavy metal salts are soluble in water and form aqueous solutions and cannot be separated very easily by physico-chemical methods, such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange, and membrane technologies because these processes are expensive and ineffective when the heavy metal ions concentration in solutions is in rang 1-100 mg dissolved heavy metal ions/L (Volesky, 1990a and Volesky, 1990b). Biosorption/ bioaccumulation are the biological methods which are attractive alternative to physico-chemical methods for the removal of heavy metal ions present in waste water. Recent research indicates that many microorganisms can accumulate large concentration of metals (Kapoor and Viraraghavan, 1995; Ramteke, 2000). Remediation of heavy metals by using microbial cells offers a potential alternative to existing methods of decontamination of heavy metals. Researchers have studied on algae, fungi and bacteria for accumulation of heavy metals and its application in the biological processes for the removal of heavy metals from wastewater and found it more effective (Kuyucak and Volesky, 1989). Heavy metal is also accumulated in marine algae and it is a continuous process in which older tissues usually contains higher levels of some heavy metals. Heavy metal concentrations dependent both on the external factors like inorganic and organic complex molecules, pH, salinity and on physico-chemical parameters like light, oxygen, temperature and nutrients which control the metabolic rate (Barreiro, *et al.,* 1993).

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Heavy metal bioremediation by algae species

Phytoremediation is a biological process in which living plants are used to remove, accumulate, degrades or cleans up the environmental contaminants. This remediation technique involves the utilization of nutrients through the plant root system and is transported by the capillary action from the soil and ground water which is natural ability of vegetation. In this process biological mechanisms of plant accumulate and reduce concentrations of inorganic and organic pollutants present in soils and groundwater, although this process is very slow process and rely on a plants growth rate. Phytoremediation has the potential to serve as a sustained, ecologically sound method to remediate contaminated soil and groundwater (Brown and Hall, 1990). Algae are more frequently used for phycoremediation process helps in cleanup the environment. Microalgal are successfully used as biosorbing agent for accumulation of heavy metals because it use light as an energy source and facilitating the maintenance of metabolism in the absence of organic carbon sources, and also electron acceptor required by bacteria or fungi (Donmez and Aksu, 2002). Biosorption using blue green algae are gaining increasing attention as they are rich source of vitamins and proteins. The biomass can absorb and adsorb metal ions from aqueous solution even when the cells have been killed. The advantages of blue green algae over the conventional methods is the process does not produce chemical sludges (i.e Non polluting), it is selective, more efficient, easy to operate and hence cost effective for the treatment of large volumes of waste waters containing low pollutant concentrations (Garnham, *et al.,* 1992).

Macrophytes play an important role in the aquatic communities as they take part in oxygen production, nutrient cycling, and controlling water quality and provide habitat and shelter for aquatic life Ravera, 2001. Macrophytes actively take up metals from the sediments through their roots and translocate them to the shoots, which are available for aquatic animals, epiphytic phytoplankton, herbivorous and detritivorous invertebrates (Cardwell, *et al.,* 2002), which represents a major route of bioaccumulation of heavy metals in the aquatic food chain. Heavy metals concentrations of Hg, Cd, Co, Cu, Mo, Ni, Pb, Tl and Zn were measured in macrophytes and water samples of five rivers namely; Gavaraget, Argichi, Makenis, Masrik each of them meeting the Lake Sevan, Armenia. The concentrations of different heavy metals were higher in macrophytes than in their respective water column, which indicates their role of the biogeochemical cycles of heavy metals.

Heavy metal detoxification mechanism

Algae are aquatic organisms that are able to discriminate between essential and non essential heavy metal ions. They maintain non-toxic concentration of these medium inside their cells. the two principle mechanism have been identified, the one which prevents the entrance of indiscriminate heavy metal ions into cell, i.e., exclusion and the other which prevents bioavailability of these toxic ions once they are inside the cell, i.e., the formation of complexes. The organisms maintain an equilibrated relation with heavy metal ions present and available in the surrounding. Cells have two tasks, the first is selection of heavymetals essential for growth and also exclude those that are not, and the second task is to keep essential ions at optimal intracellular concentrations (Cobbett and Goldsbrough, 2002). Land plants, aquatic plants and algae all have the capacity to eliminate heavy metal. Microalgae, fungi and some eukaryotic photosynthetic organisms, develop the production of peptides which are capable to bind heavy metals. To prevent or neutralize the potential toxic effects of heavy metal ions, the molecules, like organometallic complexes, are further partitioned inside vacuoles which facilitate appropriate control of the cytoplasmic concentration of heavy metal ions (Cobbett and Goldsbrough, 2002).

This mechanism used by eukaryotic cells employs ATP consuming effect of heavy metals of enzymatic change to achieve detoxification (Nies, 1999). The peptides are categories in two groups the first is the enzymatically synthesized short chain polypeptides derived from glutathione (cECG) named as phytochelatins class III metallothioneins, which are present in higher plants, algae and certain fungi and the second is the gene-encoded proteins; like class II metallothioneins, which are identified in higher plants, cyanobacteria and algae class I metallothioneins, which are present in most vertebrates, *Neurospora* and *Agaricus bisporus.*

Effect of algae species on heavy metals in fresh water ecosystem

The researchers have conducted studies on heavy metals (Cd, Pb, Hg, Cr) accumulation in water and plankton of Sarýyar Dam Lake (SDR). This study involved determination of physio-chemical parameters of water and their correlation with heavy metals. In plankton higher concentration of all heavy metals was reported. Hg was lowest where as the Pb was highest; but the concentration of each metal varied seasonally (Tahir, *et. al.,* 2008). The similar study was done to determine the accumulation of heavy metals in water and plankton (phyto and zoo) of Beysehir and Mogan Lake. Beysehir and Mogan Lakes are two shallow Lakes, interconnected hydrologically in the close vicinity of Ankara, Turkey. These lakes are under environmental protection status. The pollution state of the Lakes is attributed to the construction of a sewage system going around Beysehir and Mogan Lakes and collecting wastewater discharges and restrictions to urban settlement development around the Lakes. Potential impacts are from extensive agriculture, recreation, incomplete infrastructure and human activities, like residential settlements, are discussed with reference to previous and more recent pollution monitoring. Phytoplanktonic dominant algae

present in Beysehir Lake were Zooplanktonic dominant organisms present in Beysehir Lake were *Eudiaptomus drieshi, Daphnia longispina* and *Brachionus calyciforus* while in Mogan Lake *Arctodiaptomus* sp., *Keratella quadrata, Filinia longiseta* and *Diaphanosoma lacustris* were present. Accumulation of heavy metals (Cd, Pb, Hg and Cr) in the water and plankton of Beysehir and Mogan Lakes was studied seasonally, from April 2000 to December 2004. In this study the concentrations of all heavy metals were also high in the plankton. Mercury (Hg) was in least concentration where as lead (Pb) was in the highest concentration; however, the concentration of other metal varied seasonally. It was also determined that because of seasonal changes there is change in populations and species of phytoplankton and zooplankton (Tahir, *et.al.,* 2010).

Cladophora is also considered as a good collector of nutrients like nitrogen and phosphorus and heavy metals in water (Borovitzka and Norris, 1986). Researcher studied to assess the amount of heavy metals (Zn, Mn, Fe, Pb, Cd, Cu, Cr and Ni) in biota (mainly in filamentous of green algae *Cladophora*), water and sediment. They found that the concentration of heavy metals in sediment increases in the sequence of Pb>Cd>Ni>Cu> Cr. But in biota the concentration sequence was Cd>Ni>Cr>Cu>Pb. Heavy metals accumulation in algae is an efficient method and allows a convenient way to determine the average pollution of water. However, it depends largely on the accumulative capacity of each organism. Their average values of heavy metals like Cu, Pb, Cd, Ni and Cr in water, in sediments and in biota are given in Fig 1 (Kupe, *et al.,* 2010). The intensity of heavy metals accumulation in algae is higher than in water, as a result *Cladophora* is used as an object of monitoring (Levkov and Krstic, 2002).

Few more researcher worked on accumulation of heavy metals in *Cladophora glomerata* like, in the main stream of Wadi Hanifah heavy metal contamination was increased due to Anthropogenic activities. Some researchers determined heavy metal concentrations in the main stream, by using two filamentous green algae *Enteromorpha intestinalis* (Linnaeus) Nees and *Cladophora glomerata* (Linnaeus) Kutzing collected from three different sites. They digested the dried algae sample using appropriate acids and measured the concentration of manganese (Mn), copper (Cu), Zinc (Zn), Arsenic (As), cadmium (Cd) and lead (Pb) in the aliquot samples using Inductively Coupled plasma-Optical Emission spectrometer (ICP-OES). Manganese, copper and arsenic were detected in high concentration at all sites which was indicating a high degree of pollution by these elements. Zinc, cadmium and lead level was within the expected limits. The researcher reported that the *Enteromorpha intestinalis* can be used as an excellent indicator for manganese, zinc and arsenic pollution, whereas *Cladophora glomerata* can be used as an excellent indicator for copper, cadmium and lead pollution in this area. Serious efforts should be considered to decrease the heavy metal levels in this fragile and valuable habitat as the stream runs through the city of Riyadh (Al-Homaidan, *et al.,* 2011).

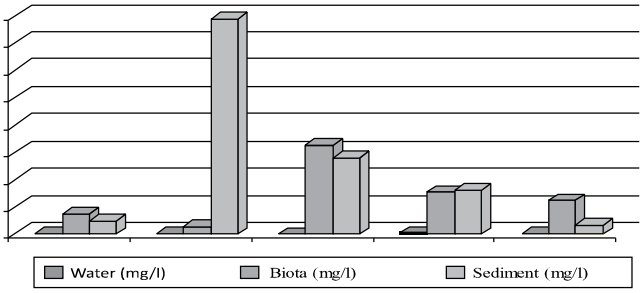

Fig. 1. Average value of Cu, Pb, Cd, Ni and Cr in water, biota and sediments (Kupe, *et al.,* 2010)

The toxicity and accumulation of the heavy metals, lead (Pb) and cadmium (Cd) was also studied in a common filamentous green alga, *Cladophora fracta*. *Cladophora fracta* were cultured in a modified Chu No. 10 medium. The toxicity symptoms of Pb and Cd to *Cladophora fracta* was significantly decreases in the relative growth and total chlorophyll content, it also damaged and disintegrated cell wall and ultimately cell death. There was significant increase of Cd and Pb metal levels in algal tissue when the exposure time and concentration were increased. The accumulation potential of *Cladophora fracta* for Pb was higher than that for Cd which indicates that bioconcentration factor (BCF) of Pb was higher than that of Cd at the same time of exposure.

The algal isolates like *Anabaena variabilis, Aulosira sp., Nostoc muscorum, Oscillatoria* sp. and *Westiellopsis sp*. were grown in 100% effluent supplemented with and without basal nutrients and in Siruvani water which was used as control. The result showed hat the Blue green algae can grow very well in polluted water. *Anabaena variabilis* performed well for biosorbing the heavy metals; the maximum biomass production was reported in the presence and absence of basal nutrients (12.47 and 10.69g /L respectively) where as in control the biomass was comparatively very less (5.54 g/L). The algae bind 40-90 percentages of heavy metals from solution in the active phase of their growth (15th day). Blue green algae cultivation promotes hyper production of mucous exopolyoses and can be used for heavy metals removal from waste water due to its high rate and capacity of adsorption and absorption (4.9 to 18.3 ppm). *Anabaena variabilis* reduced Cr, Cd, Ni and Pb to 0.67, 0.57, 1.72 and 1.32 ppm respectively in 28 days, while at 28th day 100% reduction of heavy metals was noticed in all the treatments. The results indicate that the algal culture can be effectively used in the bioremediation of wastewater for the removal of heavy metals. The algae were also able to completely remove the offensive odour of the effluent (Parameswari, *et al.,* 2010).

Effect of algae species on heavy metals in marine water ecosystem

Marine algae act as time- integrators of pollutants in benthic food webs. Researcher used eleven different marine algae species *Cystoseira barbata, Padina pavonia* (brown algae); *Ulva lactuca, Enteromorpha compressa, Cladophora vagabunda, Chaetomorpha gracilis* (green algae); *Antithamnion cruciatum, Corallina mediterranea, Corallina officinalis, Jania rubens and Pterocladia capillacea* (red algae) in their study. The accumulation of heavy metal Co, Cr, Cu, Fe, Mn, Ni and Zn was highest in marine algae species *Antithamnion cruciatum,* Cd in *C. gracilis* and Pb in *P. capillacea*. Regarding the areas the highest amounts of heavy metal Co, Cr, Cu, Fe, Mn, Ni and Zn were found in Sinop, Cd in Bulgarian Black Sea coast. Sinop was considered to be more polluted than other sites of the Black Sea. All metal levels gradually decreased during the past years when the heavy metals concentrations was tested in *C. barbata* and *U. lactuca* algae species compared to the previous study in the same species. In Marmara Sea the highest accumulation of heavy metals in marine algae species were Cd, Fe, Mn, Ni, Pb and Zn

Table 1. List of Algal Species with their heavy metal accumulation capacity in different fresh and marine ecosystem

S.No	Algal Sample	Source	Heavy Metals	Accumulation of Heavy metal	Reference
1.	*Choroococcus, Gomphospheria, Oscillatoria, Phormidium, Anabaena, Nostoc, Cystodinium, Dinobryon, Ankistrodesmus, Chlorella, Coelastrum, Oocystis, Pediastrum, Scenedesmus, Cosmarium, Oedogonium, Cyclotella, Achnanthes, Amphora, Anomoneis, Cocconeis, Cymbella, Diatoma, Diploneis, Fragilaria, Gomphonema, Navicula, Nitzschia, Nitzschia, Rhoicosphenia, Surirella* and *Synedra*	Sarıyar Dam Lake (SDR)	Cadmium, Lead, Mercury, Chromium	Lead Highest Accumulation Mercury Lowest Accumulation	Tahir, *et. al.,* 2008
2	*Oscillatoria* sp., *Cladophora* sp., *Achnanthes* sp., *Gomphonema* sp., *Navicula* sp., *Cosmarium* sp., *Cymbella* sp., *Fragilaria* sp., *Oocystis* sp., *Spirogyra* sp., *Diatomae* sp., *Microcystis* sp. and *Staurastrum* sp.	Beysehir Lake	Cadmium, Lead, Mercury, Chromium	Lead Highest Accumulation Mercury Lowest Accumulation	Tahir, *et. al.,* 2010
3.	*Spirogyra* sp., *Zygnema* sp., *Euglena* sp., *Achnanthes* sp., *Cymbella* sp., *Fragilaria* sp. *Navicula* sp., *Scenedesmus* sp., *Oocystis* sp., *Synedra* sp., *Oscillatoria* sp., *Chlorella* sp., *Cosmarium* sp. and *Nitzshia* sp.	Mogan Lake	Cadmium, Lead, Mercury, Chromium	Lead Highest Accumulation Mercury Lowest Accumulation	Tahir, *et. al.,* 2010
	Cladophora sp.	Polluted water of Lake	Cadmium, Nickel, Copper, Chromium, Lead	Cd>Ni>Cr>Cu>Pb	Kupe, *et al.,* 2010
4.	*Cladophora glomerata*	Main stream of Wadi Hanifah	Copper, Cadmium, lead	Excellent Accumulation	Al-Homaidan, *et al.,* 2011
5.	*Enteromorpha intestinalis*	Main stream of Wadi Hanifah	Manganese, Zinc, Arsenic	Excellent Accumulation	
6.	*Cladophora fracta*	In laboratory cultured medium Chu No. 10	Lead	Excellent Accumulation	Chantana, *et al.,* 2005
		In laboratory cultured medium Chu No. 10	Cadmium	Moderate Accumulation	
7.	*Anabaena variabilis*	Siruvani polluted water	Chromium, Cadmium, Nickel and Lead	Excellent Accumulation	Parameswari, *et al.,* 2010
8.	*Antithamnion cruciatum,*	Sinop Marine	Cupper, Chromium, Cobalt, Iron, Manganese, Nickel and Zinc	Highest Accumulation	Sayhan Topcuoğlu, *et.al.,* 2010
9.	*Chaetomorpha gracilis*	Bulgarian Black Sea coast	Cadmium	Highest Accumulation	
10.	*Pterocladia capillacea*		Lead	Highest Accumulation	
11.	*Ulva lactuca*	Marmara Sea	Cadmium, Iron, Manganese, Nickel, Lead and Zinc	Excellent Accumulation	
12.	*Cystoseira barbata*		Copper, Chromium and Cobalt	Excellent Accumulation	
13.	*Chlorella* sp.	Marine ecosystem	Cadmium	Excellent Accumulator	Matsunaga, *et.al.,* 1999
14.	*Chlorella* sp. and *Scenedesmus* sp.	Batch culture medium	Chromium	Excellent Accumulator	Travieso, *et al.,* 1999
15.	*P. tricornutum*		Cadmium	Moderate Accumulator	
16.	*Scenedesmus* sp.	-	Uranium	Excellent Accumulator	Zang, *et al.,* 1997
		-	Copper, Cadmium		Terry and Stone, 2002
		-	Zinc		Torres, *et al.,* 1997; Aksu, *et al.,* 1998; Travieso, *et al.,* 1999; Canizares-Villanueva, *et al.,* 2001

in *Ulva lactuca* and Co, Cr and Cu in *Cystoseira barbata* (Topcuoðlu, *et al.,* 2010).

Several species of *Enteromorpha* and/or *Cladophora* green algae have been widely used to measure heavy metals toxicity level in many parts of the world in last few years. *Chaetomorpha aerea, Enteromorpha clathrata* and *Ulva lactuca* green algae were used to measure the levels of cadmium, copper, iron, lead, nickel and zinc in three sites on the Saudi coast of the Arabian Gulf (Al-Homaidan, 2007). In the Dammam area on the Saudi coast nickel contamination level was checked in twelve different species of brown, green and red algae [Al-Homaidan, 2008]. High Rate Algal Ponds (HRAP)(Oswald, 1988) and the patented Algal Turf Scrubber (ATS) (Craggs, *et al.,* 1996 are the most common arrangements, which employes suspended biomass of cyanobacteria (*Spirulina, Oscillatoria and Anabaena*), common green algae (*Chlorella, Scenedesmus and Cladophora*) or consortia of both. To remove inorganic nutrients for the pollution control, algae-based biotechnologies are used widely (Hoffmann, 1998).

As per the survey based on batch growth of the microalgal species, various algal strains are capable to remove heavy metals. Researchers created marine environment where *Chlorella* strain was capable to sustain its growth at 11.24 mg Cd^{2+}/liter and 65 % removal when exposed to 5.62 mg Cd^{2+}/liter (Matsunaga, *et al.,* 1999). Few researchers working with *Chlorella* and *Scenedesmus* strains in the batch cultures at 20 mg Cr^{6+}/liter they found 48% and 31% removal of Cr respectively. *P. tricornutum* algae species is of high Cd6+ tolerance, which is characterized by Metallothioneins III production pattern.

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In Vitro Mass Propagation of *Scoparia dulcis* L. Through Nodal Segments

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ABSTRACT

***Scopari ducis* L., one of the highly medicinal plant found in tropical and subtropical region. Locally it is called as *"chinijhar"* or *"mithajhar"*. An effective protocol was established for mass propagaton of *Scoparia dulcis* (Family: Scrophulariaceae). The main phytochemicals studied are scopadulcic acids A and B, scopadiol, scopaduliciol, scopadulin, scoparic acids A, B, and C, and betulinic acid. An antidiabetic compound, 'amellin' has been reported in leaf and stem of the fresh green plant. Nodal explants were used for the *in vitro* mass propagation of this plant and the best result for regeneration of shoot was found 8.25 in MS medium supplemented with 1mg/l BAP and so, BAP is the best phytohormone for the regeneration of shoot than the combination with auxin. Similarly, the best result for induction of roots from shoots were found 7.25 in ½ concentration of MS medium supplemented with 0.5mg/l IBA and 12.50 in MS medium supplemented with 0.75mg/l IBA. So, IBA alone is the best phytohormone for the regeneration of shoots then combination with NAA. 75% rooted plantlets was successfully transferred and survived in natural environment.**

Key words: *Scoparia dulcis, BAP, NAA, IBA, amellin, in vitro*

Scoparia dulcis L. is a small erect, slender, rigid, perennial herb. Phytochemical screening of *Scoparia dulcis* has shown that is a source of noval phytochemicals in the flavone and terpene classification. Many of vasssournha's active biological properties, including its anticancerous properties, are attributed to these phytochemicals. The main chemicals being studied are scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A, B, and C, and betulinic acid. An antidiabetic compound, 'amellin' has been reported in the leaf and stem of the fresh green plant. Oral administration of amellin is reported to relieve symptoms of glycosuria, reduce hyperglycaemia and increase RBC count. It has also been found to be helpful in anaemia, albuminuria, ketonuria, retinitis and other complication associated with diabetes mellitus. Unlike insulin, amellin does not cause blood sugar levels to drop below normal and reduction of both blood and urine sugar occurs gradually. Amellin is reported to raise the lowest alkali reserves in diabeties and reduce iron content of serum and of acetone bodies in blood (Anonymous, 1972). Leaf of *Scopaia dulcis* is used as a cure for gastric ulcer and physical weakness. Infusion of leaves is used in fever, cough, bronchitis, diarrhea and dysentery and as a diuretic and gargle for toothache. Decoction of leaves is useful in curing kidney problems. All parts of the plant are useful as emetic. An infusion of seeds obtained by soaking them in water overnight is a cold drink.

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and threatened medicinal plants (Ajithkumar and Seeni, 1998; Prakash, *et al*., 1999). Commercial exploitation and elimination of natural habits consequent to urbanization has led to gradual extinction of several medicinal plants. Micropropagation is an effective approach to conserve such germplasm. Further genetic improvement is another approach to augment drug yielding capacity of the plant (Tejavathi and Shailaja, 1999). *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species Azad, *et al*., 2005; Faisal, *et al*., 2003; Hassan and Roy, 2005.

To our knowledge, no proper protocol is established in micropropagation of *S. dulcis* on different phytohormones for the native plant. Thus the main objective of the present study was to establish the proper protocol on micropropagation of *S. dulcis* on different phytohormones.

MATERIALS AND METHODS

The fresh and healthy seeds were collected from the roadside based Rampur campus, Chitwan (altitude: 228m, longitude: 27^{0}40'N, latitude: 84^{0}19'E). This experiment was conducted at Biotechnology lab. in Central Department of Botany, Tribhuvan University, Kirtipur. The seeds were used for the production of healthy and well sterilized *in vitro* nodal explants. The seeds were washed thoroughly under running tap water for 3 hours and then washed with distilled water. The seeds were dipped in 70% (v/v) ethanol for 1 minute. They were then surface sterilized with 1% Sodium hypochlorite (w/v) for 10 minutes, followed by 5 times rinsed with sterile distilled water in front the laminar air flow chamber. The surface sterilized seeds were inoculated on MS (Murashige and Skoog, 1962) basal medium containing 0.8% agar and 3% sucrose. The pH of the media was adjusted to 5.8 before autoclaving at 121^{0}c for 20 minutes. The cultures were maintained for 16 hour photoperiod at 24±3^{0}c under fluorescent light. The glass jars were used for seed germination (Fig. 1). The sterile nodes were used after 6 week of culture as explants for the experiment. The MS media supplemented with different concentration of BAP (6-benzylamino purine) and NAA (á-naphthaleneacetic acid) was prepared and the explants were placed vertically on the culture medium. For this, 15×150mm culture tubes were used.

Shoot proliferation from nodal explants were obtained in combination of 0-2mg/l BAP and 0-1mg/l NAA were incorporated in MS to select the best hormone for the response

of shoot induction. Number of new shoots proliferated of each culture was recorded and photograph was taken after every week of inoculation.

For *in vitro* rooting, individual shoots were excised from the proliferated shoot and implanted into half strength of MS and full strength of MS with combination of different concentration of NAA and IBA (indole-3 butyric acid). Number of roots seen in each cultured shoot was recorded and photographs were taken at the end of every week.

The rooted plants were taken out from culture tubes, washed to remove agar gel adhered to the roots and transplanted to the plastic pots with soil, sand and compost (1:1:1) for hardening. The plantlets were kept in greenhouse at 80% relative humidity, 28±2^{0}c under a 12 hour photoperiod for acclimatization. Established plants were transplanted in pots under natural conditions and the survival rate was recorded.

RESULTS AND DISCUSSION

Nodal explants of *Scoparai dulcis* L. were cultured on MS medium supplemented with various concentrations of BAP alone, NAA alone and combination of BAP and NAA for shoot regeneration. The explants were started to swollen after week and callus like structure was formed. The swollen structure starts to produce microshoots. The explants responded in all combinations of BAP and NAA but significant amount of shoots was produced in MS medium supplement only BAP. From the Table 1, the maximum number of shoots i.e. 8.25±0.54 was observed in 1mg/l BAP (Fig. 3). 1.5mg/l BAP is also suitable for the proliferation of microshoots (Table 1, Fig. 2). From the Table 1, it can be concluded that, NAA may not be proper supplement for the induction of shoots of *Scoparia dulcis* L. On increasing the concentration of NAA, the production of shoots was decresed. So, NAA may inhibit the induction of shoots at that condition. Hassan, *et al*., 2009 reported that MS medium with 1mg/l BAP is best supplements for the regeneration of microshoots from shoot tips and nodal explants.

Table 1. Effect of growth regulators in MS medium for shooting of *Scoparia dulcis* using nodal segments

Growth regulators (mg/l)			Mean number of shoots (±SE)*
BAP	NAA	BAP+NAA	
0	0	0+0	4.75±0.41
0.5	0	0.5	5.25±0.96
1.0	0	1.0	8.25±0.54
1.5	0	1.5	6.75±0.96
2.0	0	2.0	5.00±0.35
0	0.1	0	6.00±1.87
0.5	0.1	0.5	6.50±0.56
1.0	0.1	1.0	4.50±1.09
1.5	0.1	1.5	2.50±0.25
2.0	0.1	2.0	3.75±1.14
0	0.5	0	4.50±0.75
0.5	0.5	0.5	1.75±0.41
1.0	0.5	1.0	2.50±0.56
1.5	0.5	1.5	3.25±0.65
2.0	0.5	2.0	2.00±1.06
0	1.0	0	2.50±o.43
0.5	1.0	0.5	2.50±0.25
1.0	1.0	1.0	1.50±0.56
1.5	1.0	1.5	1.50±0.25
2.0	1.0	2.0	1.50±0.25

*Standard error. Results are mean±SE with 4 replications.

Roots initiated by the end of second week of culture. The maximum number of roots in regenerated shoots of *Scoparia dulcis* was achieved (7.25±0.41) in ½ MS medium supplemented with 0.5mg/l IBA (Table II, photo plate-5) within 5 weeks of culture. Similarly, roots produced by regenerated shoots of *S. dulcis* was achieved maximum number (12.50±1.25) was found at the cultured of microshoots on MS medium supplemented with 0.75mg/l IBA (Table II, photo plate-6) within 5 week of culture. So, from the above table, NAA shows less significant for the induction of roots in combination of IBA and IBA is the best supplements for the *in vitro* root generation for this condition. Use of auxins singly or in combination for rooting was also reported by different authors (Baskaran and Jayabalan, 2005; Hassan and Roy, 2005; Rahman, *et al.,* 2006; Baksha, *et al.,* 2007).

After eight weeks, the rooted shoots were transferred in plastic pots for hardening up to three weeks under semi-controlled condition of temperature (28±2^{0}c), light (12 hr photoperiod) and moisture at 80% relative humidity (Fig. 7). During this period of acclimatization, shoots elongated, leaves expanded and turns deep green. After three weeks of acclimatization, plants were transferred to an open place and gradually acclimatized to outdoor conditions (Fig. 8), where 75% plants were survived. The technique using micropropagation described here appears to be readily adaptable for large scale mass propagation and plantation for

Table 2. Effect of rooting hormones in ½ MS and MS medium for rooting of *Scoparia dulcis* using microshoots.

Growth regulators (mg/l)			Mean number of root (±SE)*	
NAA	IBA	NAA+IBA	½ MS	MS
0	0	0+0	6.25±0.96	03.25±0.69
0	0.50	0+0.50	7.25±0.41	09.00±2.50
0	0.75	0+0.75	3.25±0.41	12.50±1.25
0	1.00	0+1.00	1.75±0.41	09.25±2.69
0.50	0	0.50+0	3.00±0.94	07.75±0.69
0.50	0.50	0.50+0.50	5.00±0.61	08.75±2.19
0.50	0.75	0.50+0.75	5.75±0.74	07.75±3.69
0.50	1.00	0.50+1.00	5.25±0.54	07.00±2.50
0.75	0	0.75+0	5.25±0.41	05.25±2.69
0.75	0.50	0.75+0.50	6.75±1.43	07.50±3.25
0.75	0.75	0.75+0.75	3.50±0.90	09.50±1.25
0.75	1.00	0.75+1.00	1.25±0.22	05.50±3.25
1.00	0	1.00+0	2.75±1.08	03.00±0.50
1.00	0.50	1.00+0.50	4.25±0.96	03.25±2.69
1.00	0.75	1.00+0.75	2.75±0.41	02.75±1.69
1.00	1.00	1.00+1.00	4.00±0.79	01.75±0.19

*Standard error. Results are mean±SE with 4 replications.

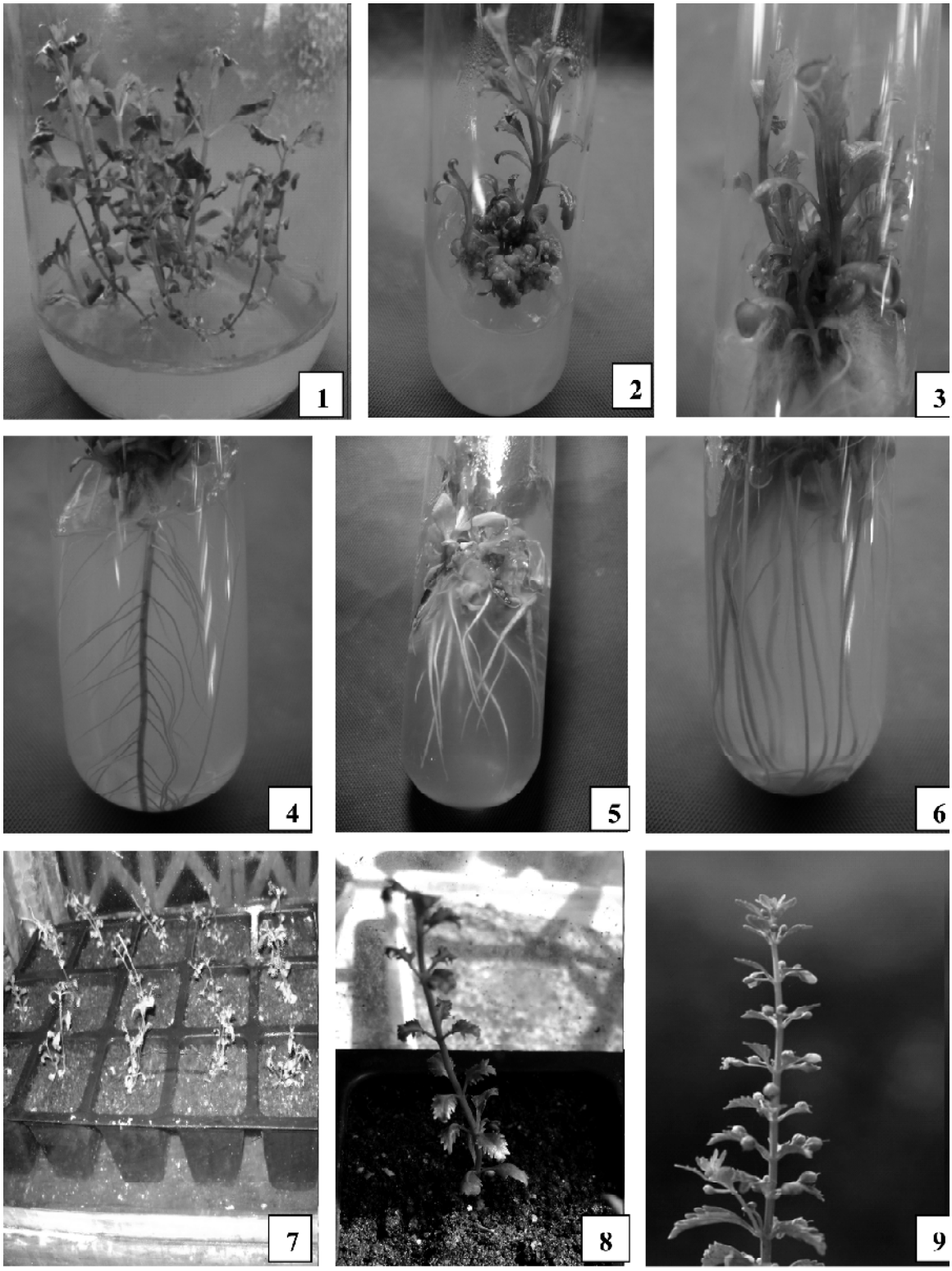

Fig. 1. *In vitro* regeneration of *Scoparia dulcis* from nodal culture. 1. Sterile plants used for nodal explants. Fig. 2. Induction of shoot in MS medium combination with 1.5mg/l BAP. Fig. 3. Proliferated shoots in MS medium with 1mg/l BAP. Fig. 4. Rooting of *in vitro* regenerated shoot cultured hormone free ½ MS medium. Fig. 5. Rooting of *in vitro* regenerated shoot cultured in ½ MS medium supplemented with 0.5mg/l IBA. Fig. 6. Induction of root in MS medium supplemented with 0.75mg/l IBA. Fig. 7. Acclimatization of regenerated plant for hardening. Fig. 8. Transfer of acclimatized plants on natural condition after 3 week. Fig. 9. Regenerated fully grown plant with flowering and fruiting condition.

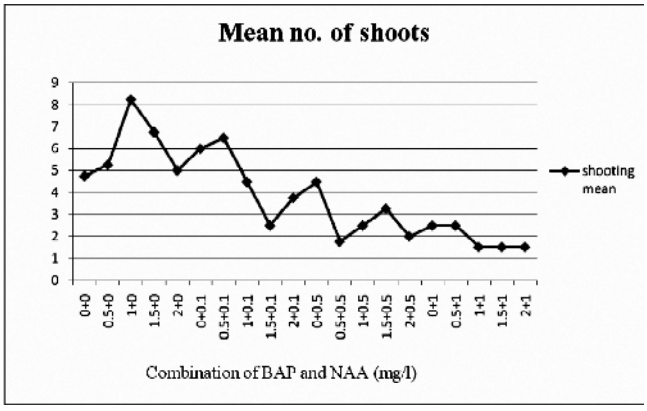

Fig. 10. Graphical representation of concentration of hormone vs mean no. of shoots

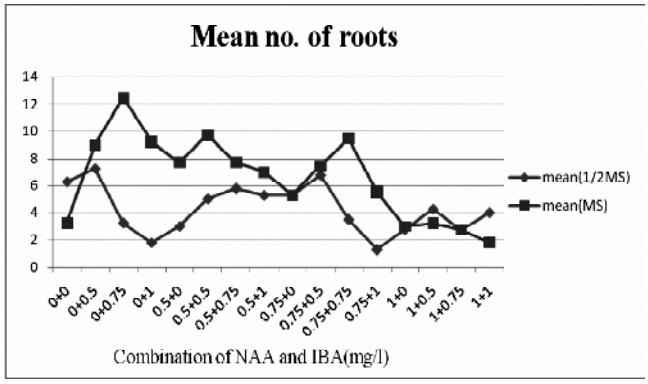

Fig. 11. Graphical representation of concentration of hormones vs mean no. of roots

sustainable use in the industry. Further, by using this protocol for mass propagation of selected plants, it is possible to achieved a tenfold increased in the products per unit area of cultivation (Hassan and Roy, 2005).

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Biomining of Copper Using Halophilic *Thiobacillus ferroxidans* N-9

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ABSTRACT

Bio mining is a process of extracting minerals from ores using microorganisms. It is a biochemical process involving interactions between microbes and minerals to recover valuable metals. It is one of the environment friendly process of mining and mineral processing.In the present study 11 different bacterial isolates were isolated from hyper saline soils of Kolhapur district. Isolates were identified using Bergey's manual of systematic bacteriology. All the isolates were investigated for bioleaching of copper using low grade ore Chalcopyrite. Of all isolates, isolate no N-9 identified as *Thiobacillus ferroxidans.*N-9 **is found to be most suitable for bioleaching of copper ore in both shake flask as well as bioreactor study. The results showed that in the shake flask the isolate no.N-9 tolerates 40 g/L of Chalcopyrite when supplemented with 0.5 G/L of yeast extract. At 120 rpm and at 40°C temperature,78% of copper can be extracted from 40 g/L of Chalcopyrite after 14 days. Bioreactor study indicated the total extraction of 85% can be achieved in 12 days. Present study indicates the usefulness of isolate N-9 in bioleaching of copper from its low grade ore chalcopyrite.**

Key words: *Biomining, Halophilic, Thiobacillus thiooxidans N-9, Chalcopyrite,copper.*

Bioleaching is a simple and effective process used for metal extraction from low grade ores and mineral concentrates using the chemolithotrophic bacteria. The extraction of copper from low grade ore is to days need because of gradual depletion of high grade ore (Olsen, *et al.,* 2003).

There are many techniques proposed to extract metals but these are not practically suitable, as these requires a very high energy input as well as most of them creates environmental pollution problem, that also rises the cost of environmental protection throughout the world (Watling, 2006).

Bio processing of mineral is the only method considered as most convincing way to solve these problems. As these processes are easy to operate, requires less energy and they are free from environmental problems and non-competitive economics of conventional methods.

The bacteria most active in bioleaching belongs to the genus *Thiobacillus* (Vishniae and Santer, 1957, Trudinger, 1967). These organisms are chemolithotrophic use iron and reduced sulphur compounds as source of energy (Mossman, *et al.*, 1999).

By keeping in view this background, in the present study Halophilic *Thiobacillus ferroxidans* N-9 is explored for bioleaching of copper from low-grade ore chalcopyrite.

MATERIALS AND METHODS

Thiobacillus ferroxidans N-9 (Fig.1) was isolated from hyper saline soil of Kolhapur district of Maharashtra, India on modified 9 K medium as per Silvermann and Lundgren, 1959. In brief composition(g/L),Solution-A: $(NH_4)_2SO_4$(3.0), KCl(0.1), K_2HPO_4 (0.5), $MgSO_4.7H_2O$(0.5), Ca(No_3) (10mg/L), 10N H_2SO_4 (1ml) Distilled water (700ml). Solution-B Chalcopyrite (40), distilled water (300ml). It was identified by using morphological, cultural, biochemical, methods as per Bergey's manual of systematic bacteriology by Williams, *et al*., 1989, and as per MICRO-IS software by Portyrata and Krichevosky, 1992.

Chalcopyrite ore was grinded to -165 / + 300 mesh (58 to 109 u) Fig. 2,3. Initial copper and iron percentage was determined by atomic absorption spectrometry as per Greenberg, *et al.,* 1992.

Tolerance: Tolerance of isolate *Thiobacillus ferroxidans* N-9 to chalcopyrite was determined by inoculating the isolate at concentrations of 10%, 20%, 30%, 40% and 50% and by incubating on shaker Fig. 4, at 40^{0}C for 48 hours Fig. 5.

Bioleaching procedure:

A standard test procedure developed by ASTM, 1990 was followed. Briefly, 2.0 g of chalcopyrite was added to 50 ml of modified 9 k medium (minus iron) in 250 ml conical flasks. Medium was sterilized at 110^{0}C for 10 minutes and was inoculated with 0.1 ml of actively growing culture of *Thiobacillus ferroxidans* N-9 at initial cell density of 1.0 *10^7 cells/ ml.Cell density was determined by Petroff-Hauser bacteria counter and as per Nephlometer standards.

Process optimization: Unless otherwise stated the experiments were carried out in 250 ml of flasks with 50 ml of modified 9 K medium. During incubation liquid samples were removed periodically filtered, centrifuged and total Cu^{++}, Fe^{++} concentration was determined by Atomic absorption spectrophotometer.

Bioleaching study was carried out in both shake flasks as well as in bioreactor.

Shake flask study

Optimization of temperature: For optimization of temperature inoculated flasks were incubated at temperatures

20^0C, 30^0C, 40^0C, 50^0C, 60^0C. For pH, at pH1.5, 2.5, 3.5, 4.5, 5.5. For Agitation at 40 rpm, 60 rpm, 80 rpm, 100 rpm, 120 rpm, 140 rpm, 160 rpm, 180 rpm, 200 rpm and 220 rpm. For yeast extract with 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0g/L, 2.5 g/L, 3.0g/L, 3.5 g/L, 4.0g/L, 4.5 g/L 5.0g/L, and 5.5 g/L. For optimization of inoculum culture was added from 1%, 2%, 3% up to 10% v/v with a cell density of 1.0 * 10^7 cells/ ml. For pulp density flasks with 9 K medium containing chalcopyrite concentration 5%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50% was inoculated with *Thiobacillus ferroxidans* N-9 with a cell density of 1.0 * 10^7 cells/ ml.

Bioreactor study: For standardization of growth and bioleaching process by *Thiobacillus ferroxidans* N-9. The parameters which were optimized on shake flask study were determined with fully automatic microprocessor controlled bioreactor model (Biostat B,B Brown international Germany) with 5L capacity.

All parameters *viz.,* Temperature, pH, Agitation, Aeration, were monitored with fully automatic device. Different parameters i.e. Inoculum size,(5% v/v), Temperature (40^0C), pH (3.5), Agitation (120rpm), Aeration (38%), and Yeast extract (0.5g/L) were kept optimum. During batch run 2 ml quantity of medium was collected after every 24 hours and analysed for growth pattern and concentrations of iron and copper.

RESULTS AND DISCUSSION

Microorganisms isolated from hypersaline soil were identified as *Arthrobacter* sp,N-1., *Bacillus* sp,N-2., *Chromobacterium* sp, N-3., *Planococcus* sp, N-4., *Pseudomonas* sp, N-5., *Micrococcus* sp, N-6., *Peptostreptococcus*, N-7., *Halococcus* sp, N-8., *Thiobacillus ferroxidans* N-9., *Halococcus* sp, N-10., *Sulfolobus* sp, N-11.

Primary analysis of chalcopyrite indicated the 32.8% copper as per Table 1.

Process optimization with respect to shake flask study indicated that the maximum bioleaching observed at temperature 40^0C., pH-3.5.,Inoculum size 5% v/v., Agitation 120 rpm., yeast extract 0.5g/L., pulp density of 15 % and time course 15 days.

Table 2 and Fig. 3 indicates the course of metal extraction during bioleaching process by Shake flask.

Shake flask study showed that there was an initial lag of 24 hours and a significant chalcopyrite leaching started after 8th days and continued up to 14th day. The rate then decreased as iron was consumed. After 14th day a total copper extraction of 78% was achieved by shake flask. Table 3 and Fig. 4 indicates course of bioleaching during bioreactor study. Results indicated that there was a lag of 24 hours as that of shake flask study, the effective leaching started after 7th day of bioleaching and continued up to 12th day of leaching process. The rate then decreased as iron was utilized. Effect of pH was studied by Silvermann and Lundgren, 1959, They observed that *Thiobacillus ferroxidans* oxidise iron optimally at pH between 3 to 3.6. My results indicated optimum leaching at pH 3.5 which are similar to that of Silvermann and Lundgren, 1989. Effect of temperature on bioleaching was studied by Ahonen and Tuovinen, 1991, they observed optimum leaching of sulphide ores by uncharacterised strain at 37^0 C. My strain gives optimum leaching at 40^oC. Effect of Chalcopyrite concentration on bioleaching of ore has been studied by several researchers, The rate of metal dissolution decreases with increase in concentration of Chalcopyrite by Deng, *et al.,* 2000, Gomez, *et al.,* 1999, Witne and Philips, 2001, Deng, *et al.*, 1999 reported the optimal pulp density 10% w/v. Gomez, *et al.,* 1999 reported 5 to 20% , Ubaldani, *et al.,* 2000 reported that there was no significant difference in rate of iron and copper dissolution at 10 to 20%. My result indicated the dissolution of copper at 15% w/v pulp density and initial pH of 3.5 at 40^oC with a particle size of 58 to 109 µ size.

Iglesias and Carranza, 1995 reported that the pre cent extraction was found to be about 90% after 12 days. Present results indicated 78% of copper can be extracted after 14 days and bioreactor study indicated the total extraction of 85% can be achieved in 12 days. Present results are somewhat similar to that of Iglesias and Carranza, 1995 results.

Kanishi and Sataru, 1992, Waksman and Joffe, 1922, isolated bacteria from soil environments and found that the bacteria from soil environments are also equally competent in

Table 1. Chemical and mineralogical analysis of Chalcopyrite

Elemental/Mineral	Composition %
Cu	32.8
Fe	26.4

Table 2. Chemical and mineralogical analysis of chalcopyrite during bioleaching. (Shake flask study)

Composition Days ⇩	Day-1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Element ⇨															
Cu^{2++}	32.8	32.0	30.2	29.4	29.0	28.2	27.4	22.2	18.2	16.3	14.1	12.2	9.1	7.2	7.0
Fe^{2++}	26.4	25.9	25.2	24.6	23.8	22.7	21.2	19.0	17.2	14.3	11.5	9.3	7.4	5.2	3.7

Table 3. Chemical and mineralogical analysis of chalcopyrite during bioleaching. (Bioreactor study)

Composition Days ⇩	Day-1	2	3	4	5	6	7	8	9	10	11	12	13
Element ⇨													
Cu^{2++}	32.8	31.6	30.2	28.8	27.3	26.4	25.1	20.2	17.4	15.3	11.4	7.1	7.0
Fe^{2++}	26.4	25.2	24.0	23.1	22.0	20.9	19.3	17.4	14.6	10.2	7.1	3.8	3.5

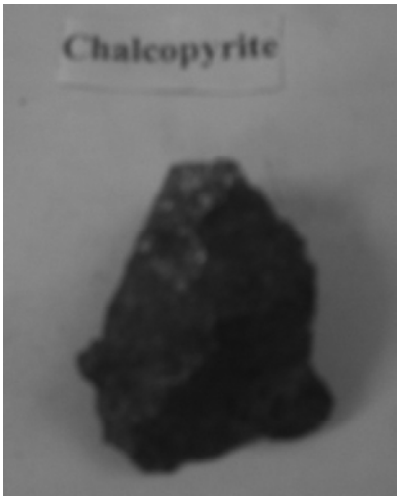

Fig. 1. Ore Chalcopyrite

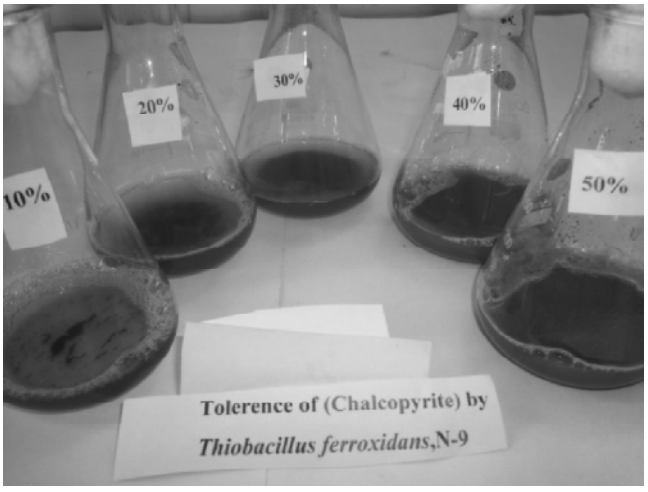

Fig. 2. Tolerence to Chalcopyrite by *Thiobacillus ferroxidans,* N-9.

leaching process. As my culture is isolated from a saline soil environment, is also have a very good leaching ability. In literature very few reports have been found on bioleaching by halophilic bacteria. Huber and Stetter, 1989, 1990 isolated two new species of halotolerent *Thiobacillus* species. *Thiobacillus prosperus* and *Thiobacillus cuprinus* from saline environment. These organisms are found to be very efficient in bioleaching of copper from Chalcopyrite. Except this information on halophilic organisms no reports have been found on use of *Thiobacillus ferroxidans* ,My report may be the opening of new era for use of Halophilic *Thiobacillus ferroxidans* N-9 as a potential candidate for bioleaching of copper from a low grade ore Chalcopyrite.

Optimum bioleaching process by *Thiobacillus ferroxidans* N-9 was observed at pH 3.5, Temperature 40^0C,Agitation 120rpm, pulp density 15%, Yeast extract 0.5g/L.

Process may be advantageous over conventional

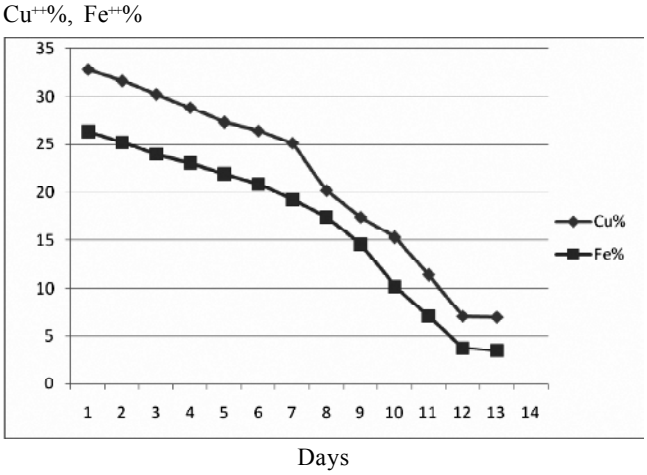

Fig. 3. Course of metal extraction during bioleaching process by Shake flask study

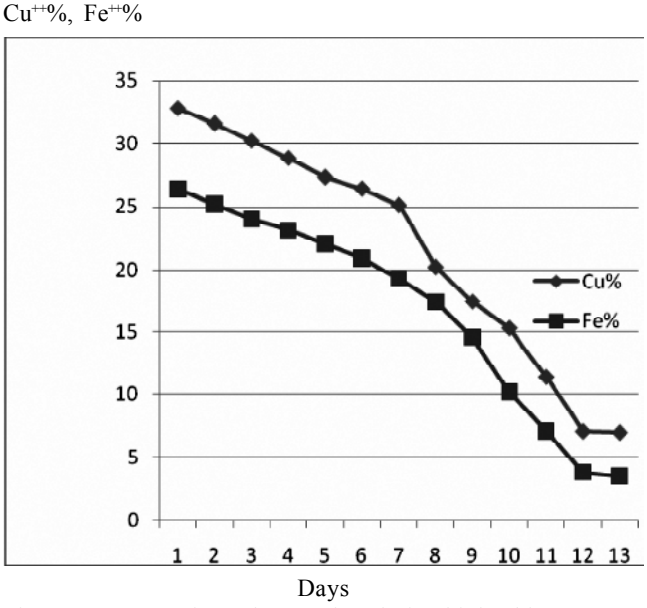

Fig. 4. Course of metal extraction during bioleaching process using Bioreactor.

method of copper extraction. Study opens promising possibilities for optimization of mining process in metallurgy industry. The isolate *Thiobacillus ferroxidans* N-9 can also be used in treatment of mineral industrial waste containing high metal concentration, which is difficult to treat by conventional methods.

Present study reports the use of halophillic *Thiobacillus ferroxidans* N-9 as a bioleaching strain.

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Impact of Processing on Metabolic Contents in the Leaves of *Stevia rebaudiana* Bartoni

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ABSTRACT

The present study was undertaken to investigate the impact of processing on metabolic contents in the leaves of *Stevia rebaudiana* and also to assess the primary and secondary metabolites under different storage conditions and durations. In this study, the plants were collected from two different centers, namely, 'Biotechnology', Bangalore and 'Jeevan herbs', Madhya Pradesh. The study indicated that the storage and preservation conditions had some impact on the primary and secondary metabolic contents in the leaves of *Stevia*. It was interesting to note that the maximum content of glycosides were recorded at 15 ^{0}C in 90 days stored leaves and least content of glycosides were observed at room temperature of 30 days stored leaves obtained from Jeevan herbs. The variation in metabolic contents of the leaves collected from two centers may be because of changes in climatic environmental factors and soil type. These results suggested that the steps need to be properly aligned during processing and exploring the natural sweetener from the *Stevia*.

Key words: *Stevia rebaudiana, storage, preservation, primary and secondary metabolites*

The worldwide demand for high potency sweeteners is increasing especially with the natural sweeteners. The natural sweeteners have become an alternative to synthetic sweetener. The *Stevia* herbs of Paraguay is capable of producing a variety of high potency low calorie sweet in its leaf tissues, thus it has become a natural sweetener. It is often referred as the sweet plant of the world. *Stevia rebaudiana* is a herbaceous perennial plant of the Astraceae family. The leaves of the *Stevia* contains zero-calorie ent-kaurene diterpeno glycosides, such as Stevioside, dulcoside, A, B, C, D, and E (Robinson, 1930, Soejarto, 1982 and Soejarto, *et al.,* 1983) that produce a sweet taste but no caloric value. The most abundant are stevioside and rebaudioside. The *Stevia* is also used as a table sugar in soft drinks, pastry, pickles, tobacco products, candy, jam, yoghurt, chewing gum, sorbets. The documented properties of *Stevia* are antibacterial, antifungal anti-inflamatory, antimicrobial, antiviral (Takahashi, *et al.,*) anti yeast, cardio tonic, diuretic, hypoglycemic.

Keeping in view, the demand for natural products the present study has been undertaken to assess the primary and secondary metabolites under different storage conditions and durations in the leaves of *Stevia rebaudiana*. The objective of the present study was not only to compare the amount of primary and secondary metabolites present in both the fresh and dried leaves, but also to understand the impact of processing conditions on the metabolic contents in the leaves of *Stevia rebaudiana*.

MATERIALS AND METHODS

The plants of *Stevia rebaudiana* Bertoni. grown in different soils and in different regions were used for the present study. The plants of *Stevia rebaudiana* grown in red soil were obtained from Biotechnology center(BC) Bangalore and the root cuttings grown in black soil were obtained from Jeevan Herbs and Agro form(JH), Madhya Pradesh. Further the plants and root cuttings obtained from BC and JH forms were potted in the department of Botany Gulbarga University, Gulbarga with red and black soils respectively. The fresh leaves of *Stevia rebaudiana* obtained from Biotechnology centre, Bangalore and Jeevan Herbs, from Madhya Pradesh were collected separately and were washed with distilled water thoroughly and gently whipped with muslin cloth. In one set of the experiment, the fresh leaves were subjected for the estimation of various metabolites and fresh leaves were shade dried in room temperature at 35^{0}C for 30 days (the experiments were carried out in April-May). These shade dried leaves were subjected for the estimation of various metabolites.

Further in another set of the experiments, the fresh leaves of *Stevia rebaudiana* of both Biotechnology and Jeevan Herbs were stored at 15^{0}C(cold dried),35^{0}C(shade dried), 42^{0}C (sun dried), 60^{0}C (oven dried) and preserved for different durations of 30, 60, and 90 days were subjected for the estimation of total proteins by Lowry's method (Lowry, *et al*., 1951) and total carbohydrates by Anthron reagent method (Yemm and Willis, 1954) and total lipids by the method of Bernad and Blackstock, 1973 and total phenols by Folin-Coicalteau method (Malic and Singh, 1980) and flavonoids by Swain and Hillis, 1959 methods, and total tannins by Folin Denis method (Schanderi, 1970) and total alkaloids by the method of Herborne and starch as per the method of Sanchez, modified by Reshi, *et al.,* 1976 and total glycosides. All the experimental triplicate results were analyzed by one way ANOVA using SPSS package (version 12).

RESULTS AND DISCUSSION

In one set of the experiment, primary and secondary metabolites in fresh and dried leaves of *Stevia rebaudiana*

obtained from Biotechnology centre and Jeevan Herbs was estimated for various metabolites (Table 1). It is observed from the results that the total content of proteins, phenols, tannins, alkaloids and sterols was found to be higher in fresh leaves and the total content of carbohydrates, lipids, flavonoids and glycosides was found to be higher in dried leaves of *Stevia rebaudiana* obtained from biotechnology center. Whereas from the Jeevan Herbs proteins, phenols, alkaloids and sterols was found to be higher in fresh leaves and the total content of carbohydrates, lipids, flavonoids, tannin and glycosides was found to be higher in dried leaves of *Stevia rebaudiana*. It is evident from results that the two plants of *Stevia rebaudiana* from biotechnology center and Jeevan herbs employed for the present study indicates that the cultivation traits showed satisfactory performance by both the plants and found that they were least affected by the abiotic factors (Megeji, *et al*., 2005). However, quantitative difference was observed in content of metabolites in both the plants. The total content of tannins was higher in fresh leaves of BC plants and was higher in dried leaves of JH plants. However the total content of glycosides was found to be higher in dried leaves of both the BC and JH plants. Similar study was carried by Hoffman and Manning, 2003 on *Stevia rebaudiana* and reported that the dried leaves were more sweet with content of glycosides than the fresh leaves.

In another set of the experiment, primary and secondary metabolites in leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology centre and Jeevan Herbs were stored in different temperatures (15^{0}C, 35^{0}C, 42^{0}C, 60^{0}C) and preserved for different duration 30, 60, 90 days (Tables 2-5).

In 30days preserved leaves: *Stevia rebaudiana* obtained from Biotechnology center was stored in different temperatures at 15^{0}C, 35^{0}C, 42^{0}C and 60^{0}C were estimated for various metabolites. It was interesting to observe that the total content of proteins was higher in cold dried (15^{0}C) leaves phenols was higher in shade dried (35^{0}C) leaves, carbohydrates, tannins, flavonoids were higher in sun dried (42^{0}C) leaves, lipids, alkaloids, sterols and glycosides were higher in oven dried (60^{0}C) leaves. Whereas from Jeevan Herbs the total content of carbohydrates, flavonoids and glycosides were higher in sun dried (42^{0}C) leaves. Proteins, phenols were higher in sun dried (42^{0}C) leaves, lipids, alkaloids were higher in oven dried (60^{0}C) leaves.

In 60days preserved leaves: *Stevia rebaudiana* obtained from Biotechnology center stored in different temperatures at 15^{0}C, 35^{0}C, 42^{0}C, 60^{0}C were estimated for various metabolites. It was interesting to note that the total content of proteins was higher in cold dried (15^{0}C) leaves, carbohydrates, flavonoids, sterols were higher in shade dried (35^{0}C) leaves, phenols, tannins, and alkaloids were higher in sun dried (42^{0}C) leaves, lipids and glycosides were higher in oven dried (60^{0}C) leaves. Whereas from Jeevan herbs the total content of proteins, carbohydrates, flavonoids, alkaloids and glycosides was higher in cold dried (15^{0}C) leaves, tannins, sterols, were higher

Table 1. Primary and secondary metabolites in fresh and dried (at room temperature) leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology and Jeevan Herbs Centres.

Biochemical parameters	Biotechnology Centre		Jeevan Herbs	
	Durations (30 days)		Durations (30 days)	
	Fresh leaves (mg/gm)	Dried leaves (mg/gm)	Fresh leaves (mg/gm)	Dried leaves (mg/gm)
Proteins	22.66±1.15	18.692±2.140	17.00±1.00	21.36±4.03
Carbohydrates	216.66±2.00	312.66±98.08	185.00±1.00	202.33±29.48
Lipids	42.00± 2.00	116.66±33.91	46.33±1.52	138.66±30.78
Phenols	97.00±1.0	57.33±9.30	84.00±1.00	44.66±2.91
Flavonoids	46.33±1.52	70.33±8.58	68.00±1.00	55.22±12.22
Tannins	03.60±0.20	03.14±1.37	02.5±2.64	03.96±2.29
Alkaloids	33.46±1.15	21.186±0.30	18.87±0.57	14.34±5.63
Sterols	82.18±2.00	57.47±2.25	53.00±1.12	44.58±0.49
Glycosides	196.66±2.00	375.34±255.55	174.99±1.00	330.50±99.95

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.01

Table 2. Primary and secondary metabolites in cold dried (15^{0}C) leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology Centre and Jeevan Herbs preserved for different durations

FARMS	Durations (in days)	PARAMETERS								
		Proteins (mg/gm)	Carbohydrates (mg/gm)	Lipids (mg/gm)	Phenols (mg/gm)	Flavonoids (mg/gm)	Tannins (mg/gm)	Alkaloids (mg/gm)	Sterols (mg/gm)	Glycosides (mg/gm)
BIO-TECH	30	24.00±1.00	285.00±1.00	134.33±0.52	41.00±1.00	73.33±2.08	1.70±1.00	20.90±0.57	48.00±1.50	444.6±10.57
	60	18.00±1.00	215.00±1.00	78.00±1.00	40.00±1.00	68.00±1.00	1.30±1.00	18.53±0.58	34.43±0.25	471.31±0.57
	90	16.33±0.57	154.00±1.00	58.00±1.00	34.00±1.00	53.00±1.00	1.2±01.00	16.19±1.52	29.70±0.26	707.97±0.57
JEEVAN HERBS	30	18.00±1.00	390.00±1.00	48.00±2.00	36.00±2.00	56.33±1.52	1.40±1.00	18.00±0.57	41.0±00.57	676.64±1.00
	60	20.00±1.00	259.00±1.00	44022±1.00	35.00±1.00	44.00±1.00	1.30±1.00	16.54±0.17	38.26±5.77	680.90±0.57
	90	14.66±0.56	184.66±0.57	41.00±1.00	34.00±1.00	34.00±1.00	1.10±1.00	15.04±0.56	36.21±0.70	826.51±0.23

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.01.

in sun dried (42^{0}C) leaves, lipids, phenols were higher in oven dried (60^{0}C) leaves.

In 90days preserved leaves: *Stevia rebaudiana* obtained from Biotechnology center stored in different temperatures at 15^{0}C, 35^{0}C, 42^{0}C, 60^{0}C, were estimated. It is observed that the total content protein was higher in cold dried (15^{0}C) leaves obtained from biotechnology centre and carbohydrates, lipids, phenols, flavonoids were higher in shade dried (35^{0}C) leaves, sterols, tannins, were higher in sundried (42^{0}C) leaves, alkaloids and glycosides were higher in oven dried (60^{0}C) leaves. Whereas, from Jeevan Herbs the total content of carbohydrates, sterols and glycosides were found to be higher in cold dried (15^{0}C) leaves. Phenols, tannins were higher in shade dried (35^{0}C) leaves. Proteins, lipids, flavonoids were higher in sun dried (42^{0}C) leaves. Alkaloids were higher in oven dried (60^{0}C) leaves. Similarly it is observed that maximum content of steviol glycosides in the leaves of *Stevia* plant before the flowering stage. Further, reported that a number of factors such as loading rate, temperature and ambient air condition affected the drying process of *Stevia* under artificial condition.

The present investigation suggests that the impact of processing conditions on the metabolic contents in the leaves of *Stevia rebaudiana*. It was interesting to note from our study that both storage and preservation conditions had some impact on the primary and secondary metabolic contents in *Stevia rebaudiana leaves*. Hence, food industrialists have to start launching new products utilizing *Stevia*. This would obviously provoke the need to grow more and finally result in more area under Stevia cultivation. Program need to be organized to promote this natural sweetener and create an awareness. This new crop would certainly give Indian farmers a choice of profitable income and healthy alternative to zero calorie sugar.

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Table 3. Primary and secondary metabolites in shade dried (35^{0}C) leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology Centre and Jeevan Herbs preserved for different durations.

FRAMS	Durations (in days)	PARAMETERS								
		Proteins (mg/gm)	Carbohydrates (mg/gm)	Lipids (mg/gm)	Phenols (mg/gm)	Flavonoids (mg/gm)	Tannins (mg/gm)	Alkaloids (mg/gm)	sterols (mg/gm)	glycosides (mg/gm)
BIO-TECH	30	18.69±2.140	312.66±98.08	116.66±33.91	57.33±9.30	70.33±8.58	03.14±1.37	21.186±0.30	57.472±2.25	375.34±255.55
	60	16.33±1.32	230.77±47.86	90.00±22.73	47.00±7.59	65.33±6.20	02.23±0.53	21.05±0.47	46.73±4.37	447.15±196.97
	90	13.8±81.166	218.00±45.85	59.0±03.12	37.33±5.83	47.66±18.54	02.10±0.53	18.79±1.73	34.90±5.51	538.44±202.44
JEEVAN HERBS	30	21.3±64.03	202.33±29.48	138.66±30.78	44.66±2.91	55.22±12.22	03.96±2.29	14.3±45.63	44.58±0.49	330.50±99.95
	60	16.55±1.81	202.33±27.23	122.11±34.52	41.33±8.60	41.88±8.60	01.66±0.48	15.42±1.70	41.34±0.30	397.07±122.266
	90	14.88±1.45	160.66±42.72	54.55±7.32	34.66±3.60	33.33±11.69	01.16±1.00	13.45±2.48	33.13±1.3	497.30±183.69

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.01.

Table 4. Primary and secondary metabolites in sun dried (42^{0}C) leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology Centre and Jeevan Herbs preserved for different Durations

Farms	Durations (in days)	PARAMETERS								
		Proteins	Carbohydrates	Lipids	Phenols	Flavonoids	Tannins	Alkaloids	Sterols	Glycosides
BIO-TECH	30	18.53±2.61	317.00±123.78	108.50±39.99	54.50±10.44	76.00±1.41	3.35±1.70	21.25±0.37	56.80±2.52	510.86±205.47
	60	16.00±1.41	199.00±5.54	88.00±28.49	49.00±8.80	68.50±5.01	2.30±0.66	21.2±60.46	48.85±3.78	533.34±187.97
	90	13.33±.816	187.50±3.93	57.00±.894	39.50±6.09	60.00±1.41	4.90±2.30	19.92±0.30	38.15±3.23	634.99±178.94
JEEVAN HERBS	30	21.54±5.04	196.50±35.61	152.00±29.59	46.50±1.04	53.83±15.19	2.25±0.60	18.08±0.61	44.76±0.38	376.61±91.27
	60	15.50±1.04	176.00±27.40	140.83±25.40	43.00±1.41	43.83±10.44	1.85±0.50	16.53±0.25	41.35±0.17	478.36±11.03
	90	15.16±1.72	144.00±43.82	58.33±5.95	33.50±3.93	38.00±10.99	1.15±0.10	15.02±0.76	32.300.02	617.45±33.82

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.01.

Table 5. Primary and secondary metabolites in oven dried (60^{0}C) leaves of *Stevia rebaudiana* Bertoni obtained from Biotechnology Centre and Jeevan Herbs preserved for different durations

FARMS	Durations (in days)	PARAMETERS								
		Proteins (mg/gm)	Carbohydrate (mg/gm)	Lipids (mg/gm)	Phenols (mg/gm)	Flavonoids (mg/gm)	Tannins (mg/gm)	Alkaloids (mg/gm)	Sterols (mg/gm)	Glycosides (mg/gm)
BIO-TECH	30	17.00±1.00	204.00±1.00	145.00±1.00	45.00±1.00	75.00±1.00	1.80±1.00	21.59±1.00	59.00±0.10	698.44±1.00
	60	15.00±1.00	194.00±1.00	114.00±1.00	41.00±1.00	64.00±1.00	1.70±1.00	20.92±1.00	45.40±0.10	704.94±1.00
	90	13.00±1.00	184.00±1.00	57.00±1.00	34.00±1.00	59.00±1.00	1.60±1.00	20.20±1.00	35.20±1.00	798.34±1.00
JEEVAN HERBS	30	17.00±1.00	164.00±1.00	179.00±1.00	46.00±1.00	40.00±1.00	1.70±1.00	18.65±8.54	44.83±0.58	293.29±1.00
	60	16.00±1.00	154.00±1.00	164.00±1.00	44.00±1.00	34.00±1.00	1.40±1.00	16.30±1.00	41.20±1.00	488.44±1.00
	90	14.00±1.00	104.00±1.00	53.00±1.00	30.00±1.00	28.00±1.00	1.10±1.00	15.72±1.00	32.50±1.00	586.62±2.89

Each value is expressed as mean ± S.D. (n=3) and statistically significant at P<0.01.

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Comparative Studies on Efficacy of Deltamethrin and Flumethrin in Bovines (Cattle and Buffalo) in Eastern Zone of Vidarbha Region

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ABSTRACT

In the ectoparasiticide trials, single application of Deltamethrin (1.25%) @3 ml water as spray was found effective against flies and lice in 20 minutes and 30 minutes respectively and in case of ticks the per cent of efficacy was 78.60% efficacy after 48 hrs of treatment. The efficacy of Flumethrin (6%) E.C. @ 2ml of water as spray was found effective against lice and flies in 20 minutes and 30 minutes respectively. In case of ticks the per cent of efficacy was 87.87% after 48 hrs of treatment. The efficacy of Flumethrin was found comparatively better than deltamethrin ectoparasiticide.

Key words: *Cattle and buffalo, Deltamethrin and Flumethrin*

The animal wealth plays a vital role in national economy. Arthropods are considered important ectoparasites of livestock as they are known to cause direct injuries etc. None of the domesticated animals are free from attack of ectoparasites. Affected animals suffer considerable annoyance and worry which are exhibited in terms of constant swinging of tail in brushing away of flies, quivering of skin, stamping of feet and continually lunging with the mouth at irritable parts of its body. The restlessness of animal leads to loss of energy and health which directly reflects in reduced efficiency of bullock, loss in milk yield of a cow etc. In height of infestations the animals lose all control and injure themselves or their owners.

In India many drugs have been tried and reported for control of either single type of arthropod. Sangwan, *et al.,* 1988 tried Deltamethrin, Diazinon and Amitraz. Singh, *et al.,* 1993 tried 0.005% concentration of Deltamethrin. Bhagherwal, *et al*., 1994 tried 25,50 and 75 ppm concentration of Deltamethrin. Maske, *et al.,* 1995 tried Ivermectin, Amitraz, Asuntol and Pestoban, Murleedharrn, *at al.,* 2002 tried fenvalerate and Deltamethrin, Muraleedharan, 2005 tried Sumicidin and Butox.

MATERIALS AND METHODS

The bovines infected with mixed arthropods parasitism were used to assess the relative efficacy of two ectoparasiticides *viz.,* Deltamethrine and Flumethrin. The herd consisting 40 animals were split in to two group A and B each with 20 animals were used for chemotherapeutic trials with drugs *viz.,* Deltamethrin and Flumethrin respectively. The animals used in efficacy trials were having mixed arthropod infestation. The efficacy of drug was assessed on the basis of observation on mortality or elimination of ticks and lice after the commencement of treatment.

RESULTS AND DISCUSSION

The efficacy of Deltamethrin (1.25%) @3 ml/lit water as spray was found effective against flies and lice in 30 minutes and 20 minutes respectively and in case of ticks the percent of efficacy was 78.60% efficacy after 48 hrs of treatment .These findings are in closed proximity with the observations of Srivastava, *et al*., 1993. The efficacy of Flumethrin (6%) E.C. @ 2ml/lit of water as spray was found effective against lice and flies in 20 minutes and 30 minutes respectively. In case of ticks the per cent of efficacy was 87.87% after 48 hrs of treatment.

In present chemotherapeutic trials, Flumethrin (6%) E.C.

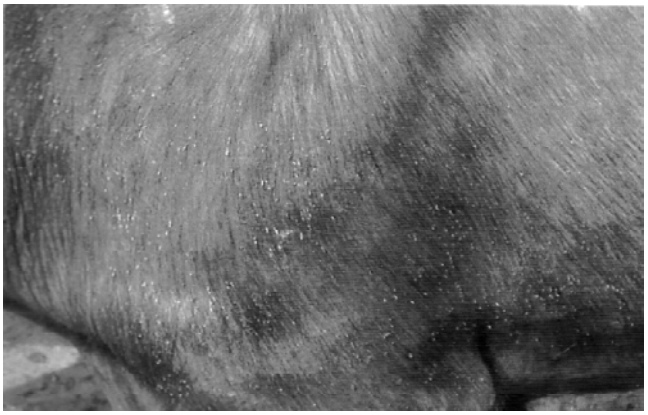

Fig. 1. Photograph of buffaloe having lice infestation

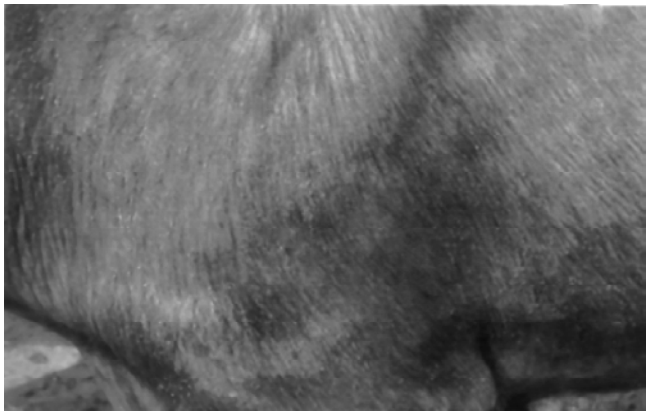

Fig. 2. Photograph after ectoparasiticide drug trial

was comparatively better than (1.25%) Deltamethrin ectoparasiticide.

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Management of Root Knot Nematode, *Meloidogyne incognita* Infecting Carnation in Commercial Polyhouse

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ABSTRACT

The experiment was conducted in nematode sick plot in farmers' polyhouse at Nelamangala, Bengaluru by using six commercially available bioagents. Among them *Paecilomyces lilacinus* treated plots recorded reduced soil and root population of *M. incognita,* number of galls/root system, number of egg masses/root system, increased plant height, root length, fresh and dry root weight, early emergence of flower bud, maximum number of flowers and increased flower yield of carnation.

Key words: *Carnation, bioagents, Meloidogyne incognita*

Carnation (*Dianthus caryophyllus* L.) is herbaceous perennial flowering plant. It is one of the most important cut flower crops in the world. Of the several limiting factors that cause serious concern to commercial production, plant parasitic nematodes are important. Although a multitude of plant parasitic nematodes are found associated with carnation elsewhere in the world (Lamberti, *et al.,* 1987), root-knot nematodes, *Meloidogyne* spp. are predominant in India (Nagesh and Parvatha Reddy, 2000). In India, yield loss in carnation due to *Meloidogyne incognita* is recorded as 26.6 per cent by Nagesh and Parvatha Reddy, 2000. A suitable management strategy is essential. For the management of nematodes expensive and hazardous chemicals are being used which cause ecological imbalance in nature. In view of this, the present study was taken up to evaluate the efficacy of different bio-agents against *M. incognita* on carnation under polyhouse condition.

MATERIALS AND METHODS

The experiments were conducted at the polyhouse of a farmer at Nelamangala Bengaluru with different biocontrol agents *viz*., *Paecilomyces lilacinus* @ 10g/m^2 ($1x10^8$ cfu/g of powder), *Trichoderma viride* @ 20g/ m^2 ($2x10^8$ cfu/g of powder), *Trichoderma harzianum* @ 20g/ m^2 ($2x10^8$ cfu/g of powder), *Pseudomonas fluorescens* @10g/ m^2 ($1x10^8$ cfu/g of powder), *Pochonia chlamydosporia* @10 g/ m^2 ($1x10^8$ cfu/g of powder), *Glomus fasciculatum* @ 20 g / m^2 ($2 x 10^8$ spores/ g of soil).

Treatments were imposed after the successful establishment of the plant in nematode sick polyhouse plots at Yelachagere, Nelamangala, Bengaluru. The plots were watered regularly whenever required. Three replications were maintained for each treatment. Carbofuran 3G was kept as a standard check. Bioagents and chemicals were applied on rhizosphere zone of the plant. Nematode population on both soil and roots were analysed at harvest. Final nematode population and flower yield were recorded at the time of harvest.

RESULTS AND DISCUSSION

The results of the trial on efficacy of bio-agents on nematode parameters *viz*., soil and root population of *M. incognita,* number of galls/root system, number of egg masses/ root system on carnation were analyzed and presented in Tables 1 and 2.

Maximum reduction of population (80.54%) was recorded in plants treated with carbofuran with a population of 115.33/ 200 cc soil. However, in bio-agents minimum nematode population 117.00 with 80.25% reduction was recorded in *P. lilacinus* followed by *G. fasciculatum* 133.33 (77.50%) and *P. chlamydosporia* 144.33 (75.65%), *P. fluorescens* 152.66 (74.24%), *T. viride* 158.33 (73.28%) and *T. harzianum* 160.33 (72.95%), respectively. Minimum nematode population 53.66/ 5g root with 78.01% reduction was recorded in *P. lilacinus* followed by *G. fasciculatum* 56.00/5g root (77.05%), *P. chlamydosporia* 58.00/5g root (76.23%), *T. viride* 59.00/5g root (75.82%), *T. harzianum* 60.33/5g root (75.27%) and *P. fluorescens* 63.00/5g root (74.19%), respectively.

P. lilacinus recorded minimum number of galls (21.66 galls/root system) which amounted 74.10% reduction (Table 1). Maximum reduction of 82.40% of egg masses was recorded (8.33 egg masses/root system) in *P. lilacinus* compared to untreated check 47.33 egg masses /root system followed by carbofuran 9.66 (79.59%) (Table 1).

Maximum plant height was observed in *P. lilacinus* (112.00 cm) followed by *G.fasciculatum* (109.60cm) Maximum root length was observed in *P. lilacinus* (33.93 cm) followed by *G. fasciculatum* (32.07). The maximum fresh root weight was recorded in *P. lilacinus* (0.89 g) followed by *G. fasciculatum* (0.76 g). The maximum dry root weight was recorded in *P. lilacinus* (0.65 g) followed by carbofuran (0.58 g) (Table 1). Early emergence of flower bud was recorded in *P. lilacinus* 99.67 days followed by *G. fasciculatum* 102.67 days. The maximum number of flowers (6.00 flowers/plant) was recorded in plants treated with *P. lilacinus*.

The reason for reduction in nematode population, galls and egg masses might be due to parasitic activity of *P.*

Table 1. Effect of bio-agents on root galling and reproduction of *M. incognita* infecting carnation under polyhouse condition

Treatments	Number of galls per root system	Per cent reduction over control	Number of egg masses/ root system	Per cent reduction over control
T1 = *Paecilomyces lilacinus*	21.66	74.10	8.33	82.40
T2 = *Trichoderma viride*	28.33	66.13	14.00	70.42
T3 = *Trichoderma harzianum*	29.00	65.33	15.66	66.91
T4 = *Pseudomonas fluorescens*	31.33	62.55	16.66	64.80
T5 = *Pochonia chlamydosporia*	27.00	67.72	12.66	73.25
T6= *Glomus fasciculatum*	24.33	70.92	12.33	73.94
T7=Carbofuran (chemical check)	20.66	75.30	9.66	79.59
T8= Untreated control	83.66		47.33	
SEm ±	0.75		0.54	
CD at 5 %	2.30		1.64	

Table 2. Effect of bio-agents on growth parameters of carnation infested with *M. incognita* under polyhouse condition

Treatments	Plant height (cm)	Root length (cm)	Roots		Days taken for appearance of first flower bud	Number of flowers/ plant
			Fresh weight (g)	Dry weight (g)		
T1 = *Paecilomyces lilacinus*	112.00	33.93	0.89	0.65	99.67	6.00
T2 = *Trichoderma viride*	107.16	27.60	0.63	0.31	107.00	3.66
T3 = *Trichoderma harzianum*	105.96	26.17	0.59	0.24	109.00	4.00
T4 = *Pseudomonas fluorescens*	103.56	22.63	0.52	0.22	110.00	3.33
T5= *Pochonia chlamydosporia*	108.33	29.17	0.73	0.37	104.67	4.00
T6= *Glomus fasciculatum*	109.60	32.07	0.76	0.49	102.67	4.33
T7=Carbofuran	113.66	28.80	0.84	0.58	99.00	5.00
T8= Untreated control	98.76	18.33	0.27	0.15	127.00	1.33
SEm ±	0.66	0.71	0.02	0.016	0.86	0.50
CD at 5 %	2.02	2.15	0.05	0.049	2.62	1.51

lilacinus on eggs and all stages of nematodes. Spores of the *P. lilacinus* also adhere to the cuticle of vermiform stages of the nematodes as they migrate through the soil. The spore germinate, penetrate the cuticle and engulfs the nematode. The hyphae of the *P. lilacinus* can also enter the nematode through body openings, such as the anus and vulva. The developing *P. lilacinus* kills the nematode by feeding on its body contents. In effect, the *P. lilacinus* acts as a parasite on the nematode. *P. lilacinus* could be used for control of nematodes. The above results with respect to *P. lilacinus* in minimizing the galls and egg masses are in conformity with the findings of Nagesh, *et al.,* 1998.

The reason for increased plant growth, yield and other parameters observed here could be attributed to the release of growth promoting substances by bio-agents (Baker, *et al*., 1986) or by producing toxic metabolites which inhibit nematodes and exclude other deleterious microorganisms. The result obtained in current investigation uphold the results observed by, Nirmal Johnson, 2000, Rao, 2007 who observed increased growth and yield of carnation and crossandra in polyhouse and field experiments by the inoculation of *P. lilacinus*, *P. fluorescens* and *P. chlamydosporia*.

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Correlation Studies on Seed Yield and Seed Quality Parameters of Broccoli (*Brassica oleracea* var. *italic* L.)

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ABSTRACT

Correlation is a powerful tool to study the character association and therefore it is very useful to facilitate selection for improvement of important characters without sacrificing the gain of other characters. The correlation study revealed that the seed yield of broccoli exhibit highly significance and positive correlation with days to maturity, plant height, number of secondary branches per plant, number of siliqua per plant, length of siliqua, and number of seeds per siliqua. Seed yield also showed significant positive correlation with number of primary branches per plant and diameter of siliqua. The highly significant and positive correlation of vigour index-É was obtained with 1000-seed weight, standard germination, seedling length and seedling moisture content while the vigour index-ÉÉ showed highly significant and negative correlation with seedling moisture content, 1000-seed weight and vigour index-É.

Key words: *Broccoli, siliqua, seedling, vigour, seed quality*

Broccoli is the member of Brassiceae family. It is developed from leafy Brassica forms, commonly known as 'Calabrease Broccoli'. It is a highly nutritious crop, rich in vitamins and a good source of sulphoraphane a compound associated with reducing risk of cancer (Choudhury, 1983). The vegetable seed production is very important and profitable enterprises but to obtain the good quality seeds, the knowledge of appropriate transplanting date required to raising the crop is essential (Jana and Mukhopadhyay, 2003). To make effective selection for higher seed yield and quality parameters, a through understanding of these characters, interrelationship among themselves is necessary. Selection for one component can bring about a simultaneous change in other.

The seed yield and quality of broccoli is complex characters, which is associated with several other characters. The varying transplanting dates have a direct response on the growth, development, seed yield and quality of broccoli. Available information on this aspect is meagre. Hence, correlations among different characters of broccoli under varying dates of transplanting were computed to understand the nature and extent of character association.

MATERIALS AND METHODS

An experiment was carried out at the Research block and Seed Testing Laboratory, Department of Seed Science and Technology, G.B. Pant University of Agriculture and Technology, College of Forestry and Hill Agriculture, Hill Campus, Ranichauri, Tehri Garhwal (Uttarakhand), during winter season of 2008-09. The treatment comprises five dates

Table 1. Correlation coefficient seed yield and seed yield contributing characters.

S. No.	Characters	Days to maturity	Plant height (cm)	No. of primary branches/ plant	No. of secondary branches /plant	No. of siliqua/ plant	Length of siliqua (cm)	Diameter of siliqua (mm)	No. of seeds/ siliqua	Seed yield/ plant (g)	Seed yield/ha (kg)
1	Days to maturity		0.750**	0.497*	0.713**	0.824**	0.567**	0.394	0.442*	0.920**	0.920**
2	Plant height (cm)			0.836**	0.855**	0.871**	0.690**	0.241	0.614**	0.725**	0.725**
3	No. of primary branches/plant				0.771**	0.708**	0.634**	0.252	0.601**	0.497*	0.497*
4	No. of secondary branches/plant					0.896**	0.803**	0.210	0.677**	0.731**	0.731**
5	No. of siliqua /plant						0.872**	0.435*	0.700**	0.839**	0.839**
6	Length of siliqua (cm)							0.416*	0.872**	0.659**	0.659**
7	Diameter of siliqua (mm)								0.280	0.446*	0.446*
8	No. of seeds/ siliqua									0.558**	0.558**
9	Seed yield/ plant (g)										1.000**
10	Seed yield/ ha (kg)										

*, ** = Significance at 5% and 1% probability level, respectively.

of transplanting *viz.,* 30th September, 10th October, 20th October, 30th October and 9th November. The experiment was laid out in Randomized Block Design with five replications. Thirty days old seedlings were transplanted in the entire experimental plot at 50 cm X 50 cm^2 spacing between plant to plant and row to row. The recommended cultural practices were given as that of a commercial crop. All observation that is directly or indirectly related to seed yield (days to maturity, plant height, number of branches per plant, number of siliqua per plant, seeds per siliqua, length of siliqua and seed yield per hectare etc.) was taken. The seed yield was recorded on net plot basis while seed yield contributing characters were recorded by randomly selected five plants from each plot.

The germination test was done on the basis of the top of the paper methods as per ISTA rule. The 100 seeds were taken for germination test in each replication with four replications in each treatment. The seedlings root length (cm), seedlings shoot length (cm), seedlings fresh weight (g) and dry weight (g), were recorded by ten randomly selected seedlings from each replication. Vigour index-É was calculated by multiplying standard germination percentage with seedling length and Vigour index-ÉÉ was calculated by multiplying germination percentage with seedling dry weight. The correlation study was carried out between seed yield and yield contributing characters and among the seed quality characters.

RESULTS AND DISCUSSION

The character associations among different morphological characters were computed to know the dependency of one character on other character (Table. 1). The seed yield showed highly significant and positive correlation with days to maturity (0.920**), plant height (0.725**), number of secondary branches per plant (0.731**), number of siliqua per plant (0.839**), length of siliqua (0.659**) and number of seeds per siliqua (0.558**). The number of primary branches per plant (0.497*) and diameter of siliqua (0.446*) also showed significant and positive correlation with seed yields. Similar results were also reported by Gill, *et al*., 1977 and Mishra, 1989. These finding shows that if seed yield contributing characters like days to maturity, plant height, number of branches per plant, number of siliqua per plant, length of siliqua, diameter of siliqua and seeds per siliqua etc. increases the seed yield of broccoli will also increases.

Character association may help in the selection of the traits associated with the highest expression in terms of quality and quantity (yield). If the association is considerably positive, it will accelerate the rate of qualitative and quantitative progress while correlation in negative direction will retard the genetic progress. The standard germination showed highly significant and positive correlation with seedling length (0.822**), seedling fresh weight (0.745**), seedling dry weight (0.624**), seedling moisture content (0.593**), 1000-seed weight (0.758**) and vigour index-É (0.696**). This result was in agreement with finding of Ludder and Burrs, 1979. The study also reviled that the seedling moisture content and seedling dry weight was showed non significant association with each other. Highly significant and

Table 2. Correlation coefficient among seed quality characters.

S. No.	Characters	Germination first count (%)	Standard germinatin (%)	Seedling shoot length (cm)	Seedling root length (cm)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling moisture content (g)	Vigour index-I	Vigour index- II	1000-seed weight (g)
1	Germination first count (%)		0.481*	0.686**	0.270	0.545**	0.389	0.382	0.313	0.440*	-0.126	0.454*
2	Standard germination(%)			0.640**	0.728**	0.822**	0.745**	0.624**	0.593**	0.696**	-0.199	0.758**
3	Seedling shoot length (cm)				0.438*	0.834**	0.554**	0.634**	0.517**	0.711**	-0.280	0.739**
4	Seedling root length (cm)					0.840**	0.774**	0.689**	0.578**	0.649**	-0.138	0.728**
5	Seedling length (cm)						0.801**	0.715**	0.714**	0.852**	-0.336	0.905**
6	Seedling fresh weight (g)							0.626**	0.791**	0.672**	-0.233	0.772**
7	Seeding dry weight (g)								0.198	0.317	0.314	0.520**
8	Seedling moisture content (g)									0.907**	-0.778**	0.869**
9	Vigour index-I										-0.771**	0.942**
10	Vigour index-II											-0.605**
11	1000-seed weight (g)											

*, ** = Significance at 5% and 1% probability level, respectively.

positive correlation was observed between vigour index-É and standard germination (0.696**), seedling length (0.852**), seedling moisture content (0.907**) and 1000-seed weight (0.942**). These observations (Table 2) were supported by Maloo, *et al*., 1990 and Mahla, *et al*., 2003. The vigour index-ÉÉ showed negative and non-significant correlation with germination first count (-0.126), standard germination percentage (-0.199), seedling length (-0.336) and also showed highly significant and negative correlation with seedling moisture content (-0.778**), vigour index-É (-0.771**) and 1000-seed weight (-0.605**).

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Study on the Influence of Fly Ash on Heterotrophic Activities of Micro-organisms in Different Soils of Kanpur

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ABSTRACT

The present study was conducted to investigate the influence of fly ash on heterotrophic activities of micro-organisms in two different soils-A sandy loam alluvial soil and an acidic soil. During this study carbon dioxide evolution was chosen as an indicator of soil microbial activities. It was also undertaken to evaluate the effect of fly ash incorporation to soil on soil-microflora with special reference to fixation and cycling of nitrogen. The incorporation of fly ash into both kind of soils (sandy loam alluvial soil and acid soil) may improves soil property. It enhances the trace metal content when soil has a poor content of such elements. It also affects the pattern of carbon dioxide evolution.

Key words: *Heterotrophic, micro-organism, fly ash, trace metals, soil-microflora*

Suspended solid particles clog the pores of soil, cause soil sickness and hamper soil fertility. The industrial soil waste particularly fly ash being highly enriched in metal elements may bring about several changes in soil property including its physic-chemical nature together with microbial activities and soil respiration. This study, therefore aims to determine the effect of fly ash incorporation to soil on the changes in soil microbial population and their activities in response to fly ash amendment with an aim to understand to governing mechanism which help to use fly ash confidently in agro ecosystem for improving to soil property and benefit crop productivity. Wong and Wong, 1986 reported that effect of fly ash on soil microbial activity.

Soil respiration has long been used to asses soil microbial activities is often defined as the uptake of oxygen or release of carbon dioxide by bacterial, fungal, algal and protozoan cells in the soil including the exchange of gasses that result from both aerobic and anaerobic metabolism. In the present study carbon dioxide evolution was chosen as an indicator of soil microbial activities. It has been widely accepted by many soil microbiologist for the evolution of the toxic effect of environmental pollutants (Muskett and Jones, 1981.

MATERIALS AND METHODS

Fly ash used in this study was obtained as produced material from a 280 MW capacity coal-fired power generating plant located at Panki, Kanpur (26 '22' E longitude) U.P., India. This power plant burns about 650 tons of coal per day yielding about 105 tones of fly ash daily.

A series of field and laboratory trails were conducted to evaluate the fate of fly ash as an amendment to arable soils. The following two types of arable soils were used for detailed experimental study.

Sandy loam alluvial soil of upper Gangetic plane- The soil was chosen because it has more or less neutral ph ranging between 7.0 to 7.5. It was obtained from moderately well drained crop field from the agricultural farm of vegetable research center of C.S. Azad University of Agriculture and Technology, Kanpur. This soil can be taken as representative soil of upper Gangetic plain. The main parameters of this soil were ph 7.3; sand 65%, silt 20%, clay 15% , organic matter 1.0%, N 0.06%, P 0.03%, K 0.02%, Ca 0.06% B 8μg^{-1}, Cu 5.0 μg^{-1}, Mn 4.1 μg^{-1} and Zn 2.1 μg^{-1}.

Acidic soil of *tarai* belt- This soil was taken because it is slightly acidic in nature with ph of 6.25, slightly unfavorable for the plant growth. It has obtained from the cultivated field at rooting depth of 0-30 cm. From Pantnagar and Haldwani district of Uttar Pradesh at the foot hill of the Himalayas. This soil may be taken as representative soil type of entire *tarai* belt in U.P. This soil has been derived from ancient sedimentary rocks of Himalayas owing to intense weathering and erosion. The soil is heavy textured and free from bases. It contains N 0.05%, P 0.02%, K 0.03%, Ca 0.03%, B 3.2μg^{-1} , Cu 2.1 μg^{-1}, Mn 4.0 μg^{-1} and Zn1.9 μg^{-1} . The soil appears to be deficient in N,P,K and B.

Microbial activities and Soil respiration- Ash: soil mixture was prepared using 0, 2.5, 5, 10 and 25% fly ash on a dry weight basis. Duplicate sample of ash : soil mixture of 200g were weighted into 500 ml conical flask. Distilled water used to moisten the ash : soil mixture to field capacity.

A simple respirometre was set up as in Fig. 1. Carbon dioxide evolved during the incubation period was trapped in 10 ml of 1M NaOH contained in a small specimen tube inside the respirometre flask connected by a thin cotton thread. The respirometre was tightly capped with a rubber stopper and incubated at a light/dark cycle of 10/8h at temperature of 25±3^{0}C. All respirometre were arranged randomly on a bench inside the culture room.

Carbon dioxide trapped in sodium hydroxide (NaOH) was removed from the respirometre flask at days 7,10,20,30 and 40 during the six week period and new specimen tubes containing NaOH were inserted. The alkali taken ere diluted to 25 ml with carbon dioxide free deionized, distilled water (prepared by boiling the deionized distilled water to get rid of

all carbon dioxide). The released carbon dioxide was determined by titrating with 0.1M standard hydrochloric acid fir total alkali after absorption in NaOH and excess alkali after precipitation with Barium Chloride (Birch and Friend,1956). Titrations were performed periodically at following intervals: 7,10,20,30 and 40 days carbon dioxide (micrograms per gram of soil) released was calculated

Data of cumulative carbon dioxide and rates of CO_2 evolved at each prescribed period were subjected to one way analysis of variance. Regression analysis of the subjected to one way analysis of the relationship between incubation period (days) and the cumulative volumes of CO_2 evolved each day from each treatment were calculated. All statistical data were computed using the method given by Sendcor and Cochran, 1967.

Microbial Population – A study was under taken to evaluate the effect of ash incorporation to soil on soil-microflora with special reference to fixation and cycling of nitrogen (*Azotobacter, Nitrosomonas* and *Nitrobactor*). The methods used to achieve selective growth of four microbial groups are given in Table 1.

The methods used to achieve selective growth of four microbial groups are given in Table 1. All plates and tubes were incubated at 28^0 C and provided with adequate moisture in the incubation environment.

RESULTS AND DISCUSSION

Many industrial waste which are highly enriched with metal elements are potentially toxic to microbes (Ranby, *et al.,* 1978). Fly ash represents one of such wastes. Hence, if fly ash is to be considered for land application it is necessary to evaluate its potential effect on the microbe-mediate ecological processes which maintain the fertility and productivity of biospheres. According to Bi, *et al.,* 2003. Growth and nutrient uptake of rabuscular micorrizal maize in different depths of soil overlying coal fly ash.

Carbon dioxide Evolution Pattern- Respiratory activities in sandy loam alluvial soil were significantly depressed ($P<0.05$) after ash amendment at all levels at each prescribed period. For sandy loam soil the maximum rate of carbon dioxide evolution rate was recorded on the fifth day of incubation followed by a substantial decline reaching to minimum at the end of incubation. Initial carbon dioxide evolution rate was found to decrease with corresponding increase in the amount of fly ash incorporated in soil. A significant difference, however, in the evolution of carbon dioxide rate was noted for the soil incorporated with fly ash at the rate of 10% above of soil. Further increase in ash treatment i.e. 25% and above depressed microbial respiration significantly but the effect diminished with incubation period. However, at 25% ash amendment rate depression was so serious that soil respiration was inhibited throughout the incubation period. In related studies Baltrus, *et al.,* 2001 reported that the role of unburned carbon in AEA adsorption as measured by Foam Index and UV-Vis method, 2001.

In the fly ash amended acid soil, depression was also observed, but this was only significant ($P<0.05$) at 10% and 25% ash addition and there was a marked significant increase at low rate of 2.5 to 5% ash application rate with respect to the control (0%). The evolution pattern of CO_2 with 2.5 and 5% fly ash addition were similar to that of the control soil but the values were slightly higher after 10^{th} day of incubation period. Two distinct phases were observed, an initial build-up phase followed by a decline phase. Peak evolution for 2.5 control and 5% treatment was recorded at 10^{th} day of the incubation period. Peak evolution of 2.5% and 5% ash amended acid soil were also higher than that of the control while 10% and 25% showed a significant depression in CO_2 evolution during the initial build-up period also. The decline phase of 10% and 25% ash amended acid soil was very rapid and was always maintained at a lower level than that of the control. In related studies Suhtter and Fuhrmann, 2001 reported soil microbial community responses to fly ash amendment.

In general, two distinct decomposition patterns can be which reflect the change in degradability of organic matter after ash amendment. The first represents the most common type observed in control in normal soils and initial build-up period, followed by decline phase (Sorensen, 1972; Tate, 1973;

Table 1. Method used for selective growth of micro organism

Microbial Group	Medium	Incubation Time
Total aerobic	Soil extract agar	10 days
Heterotrophic bacteria	Littman oxgall agar + 30 µg	6 days
Total fungi	Spreptomycin/ml	10 days
Actinomycetes	Dextrose-nitrate agar	20 days
Azotobacter spp.	Jensen´s medium	21 days
Nitrobacter spp.	Nitrate-calcium Carbonate	25 days
Nttrosomonas spp.	Medium	
	Ammonium-calcium	
	Carbonate medium	

Table 2. Logistic regression equations on the relationship between incubation days (X) and cumulative volume of daily carbon dioxide evolved (Y) for each treatment.

Regression equation	r*	F value
Sandy soil		
$Y_0 = 5.43 \log_{10} \times -0.76$	0.99	299.47
$Y_{2.5} = 4.31 \log_{10} \times -2.01$	0.91	27.03
$Y_5 = 1.36 \log_{10} \times -0.87$	0.91	29.54
$Y_{10} = 0.69 \log_{10} \times -0.39$	0.94	44.30
Acid soil		
$Y_0 = 16.62 \log_{10} \times -5.95$	0.99	1514.84
$Y_{2.5} = 119.69 \log_{10} \times -7.32$	0.99	1007.76
$Y_5 = 22.93 \log_{10} \times -14.15$	0.96	93.21
$Y_{10} = 2.06 \log_{10} \times - 1.39$	0.89	20.17

*All significant at the 0.1% level.

Y_o, $Y_{2.5}$, Y_5, Y_{10} mean 0%, 2.5%, 5%, 10% ash amendment respectively.

Kanamori and Yasuda, 1979). In present study both 2.5% and 5% ash amended acid soils exhibited this pattern. The initial increase in CO_2 evolution might be due to the moistening of soil to field capacity, which activated the soil microbial activity. Siddiqui and Singh, 2005 reported that effect of fly ash on *Pseudomonas striata* and Rhizobium on the reproduction of nematode *Meloidogyne incognita* and on the growth and transpiration of pea.

A marked initial build-up phase was recorded for the control treatment of the acid soils also and there was a rapid decline, suggesting that the soil was less resistant to microbial decomposition than the sandy loam alluvial soil. As indicated in the soil analysis data sandy loam soil contained higher content of organic matter (indicated by total organic carbon) than the acid soil. Clay protects organic matter from microbial attack (Sorensen,1972) and the protection can be long lasting. In addition, the high total carbon content of the sandy loam alluvial soil may be responsible for the higher amount of CO_2 evolved over a long period as compared with the acid soil. It has been reported that amendment of saw dust to soil gradually increase CO_2 production as compared with control soil. Bernoux, *et al.,* 2003 reported that CO_2 emission from liming of agricultural soils in Brazil.

Amendment of 2.5 and 5% fly ash from the acid soil enhanced the decomposition rate of soil organic carbon as compared with the control. The amendment of fly ash from the acid soil enhanced the decomposition of soil organic carbon as compared to the control. The amendment of the fly ash thus might initiate soil microbial activity in soil can be regained.

The decline in CO_2 evolution after peak evolution could be due to substrate exhaustion in soil, especially carbon and nitrogen, so that the microflora is then forced to utilize organic matter more resistant to microbial decomposition. Humified organic substances can be formed which are more resistant to decomposition. It could alternatively be due to the deficiency of one or more of the nutrient elements necessary to maintain the initial high rate of microbial respiration. Truter, 2002, reported that the influence of a fly ash-biosolid mixture on chemical soil properties. Use of waste products to enhance plant productivity on acidic and infertile substrates.

The second type of decomposition pattern was characterized by the occurrence of marked continued inhibition, sometimes followed by a slight increase. No obvious peak of CO_2 evolution was recorded. The low decomposition rate must be due to the toxic effect of fly ash which rendered these processes inactive. The increase inhibition with time may be due to the increased ability of some types of heterotrophs to some extractant to become adapted later in incubation.

Total Carbon dioxide Production- The high ph and electrical conductivity of fly ash have been suggested to be important elements limiting microbial activity. Correlation between total CO_2 production, ph and electrical conductivity of soil before incubation were calculated (Table 2). Significant negative correlations were obtained for both type of soils. Rippon and Pulverized fly ash aged, micro-organism colonization was found to have increased which would fit in with the present findings recorded during this study. The highly enriched trace elements in fly ash might also be potentially toxic to micro-organisms. Most of the trace elements in fly ash exist in concentrations higher than in the soil. Numerous investigations have indicated the toxicity of trace elements (Cd, Cr, Znetc.) on soil micro-organisms.

Regression analysis was used to compare the affects of fly ash on CO_2 evolution (Table 2). All regression analysis of CO_2 evolution were significant at the 1% level. Moreover, regression coefficients of sandy soils were all significantly different at the 5% confidence level (Table 2), increasing ash application depressing the production of CO_2. In addition, total CO_2 production was found to be significantly ($P<0.05$) different between all treatment groups as indicate by Duncans multiple range test (Table2). The effect of fly ash was most obvious at the 10% and 25% levels with depression of five and ten times, respectively.

On the other hand regression analysis of cumulative CO_2 production for the acid soil indicated that fly ash only depressed CO_2 production significantly at the 10% level and above, with significantly no difference in ($P<0.05$). No significant difference was found between the 3% and 6% levels. Results from total CO_2 for 10% and 25% being about three times and six times that of the control.

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Match Industry Effluent Induced Stress-ameliorating Efficacy of *Sargassum wightii* on Reduced Morphometric, Biochemical and Enzymatic Characteristics of *Cyamopsis tetragonoloba* Taub.

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ABSTRACT

The Match industry effluent containing heavy metals that are toxic to plants, reduces the plant yield and even the soil fertility, although these toxic substances could be removed by plants and dried plant materials. The ameliorative effect of *Sargassum wightii* at different concentration *viz.*, 2g, 4g and 6g/l in 60% concentrated Match industry effluent treated on *Cyamopsis tetragonoloba* Taub. was analyzed. Impact of effluent was understood by a steep decline in growth characters, pigment content and other biochemical characteristics. The powder of *Sargassum wightii* in 60% concentration of the effluent in different concentrations brought about considerable increase in germination percentage, growth and biochemical characteristics in *Cyamopsis tetragonoloba* Taub than when they were treated with Match industry effluent alone. Seaweed extract used in this study was found to be efficient in nullifying the toxicity of effluent on growth and biochemical characteristics of *Cyamopsis tetragonoloba* Taub.

Key words: *Bioadsorption, Bioremoval, Seaweeds, Match effluent*

Match industry effluents contain especially inorganic content and colour, which impart objectionable colour to the water bodies. Presence of colour reduces the light penetration and photosynthetic activities of water bodies, some of the toxic metals used in the match industries are found to be carcinogenic Roshan, *et al.,* 2000. These waste effluents can be treated by treatment methods like ozonation, chemical coagulation, adsorption and electrochemical technology. These chemical treatment methods require costly chemicals and also generable hazardous sludge, so use of plants to degrade, assimilate, metabolize or detoxify contaminants is cost effective and ecologically sounds for the restoration and management of our natural water resources. Whenever these industrial effluents are released through drainage from the nearby industries without proper treatment they reach agricultural area and make major havoc to the entire environment of the town Ramasubramanian, *et al.,* 1988.

None of the technology had so far been introduced to allay the apprehension of farmers, that effluents are reducing yield and wrest the farming from them. In the present study on attempt has been made to nullify the problem in green way i.e., by the use of seaweeds, Bioadsorption technique – using of weed plants to reduce the toxicity which present in the effluents and render them harm less to plants. This will be effective in bringing new resources and technology to solve environmental problems in India generated by industries.

There is an urgent need to apply bioadsorption technology, using dried natural algal biomass to decontaminate the polluted water bodies in the world. This will be effective in bringing new resources and technology to solve environmental problems in India, generated by industries Selvarathi, *et al.,* 2010.

This study aimed to analyze the characterization of Match effluent, the effect of Match effluents on morphometric, biochemical and enzymatic characteristics of *Cyamopsis tetragonoloba* Taub. and also study the effect of varying amount of dried natural biomass of *Sargassum wightii* with 60% effluent on the morphometric, biochemical and enzymatic characteristics of *Cyamopsis tetragonoloba* Taub.

MATERIALS AND METHODS

The Match industry effluent was collected from the Match industry in Sivakasi. The sample for analysis was preserved as per the standard recommended procedure. The seaweed *Sargassum wightii* collected from Harberpoint coast near Tuticorin were shade dried and finally powdered by milling. Various concentrations of seaweed powder was prepared with 60% Match industry effluent.

Both control and experimental seeds were allowed to grow inuniform mixed red, black and sandy soil in 1:1:1 ratio. After ten days, seedling of *Cyamopsis tetragonoloba* Taub. were treated with various concentration of Match industry effluent (20%, 40%, 60%, 80% and 100% v/v). After ten days of effluent treatment, various morphometric, biochemical and enzymatic characteristics were analyzed. In another set 60% of effluent (the concentration at which toxicity was found to be optimum level based on LST analysis). was subjected to various concentration of seaweed (*Ulva lactuca*) powder (2g/L, 4g/L and 6g/L w/v), for 24 hours. Then filtered and the filtrate was used to treat plants. After ten days of treatment, various morphometric, biochemical and enzymatic characteristics were analyzed.

Twenty days old plants of *Cyamopsis tetragonoloba* Taub. were used for measuring the morphometric characters such as root length, shoot length, leaf area, fresh weight and

dry weight were measured. The biochemical and enzymatic characters were analyzed by the following methods: chlorophyll and carotenoids Wellburn and Lichtenthaler, 1984, anthocyanin Swain and Hills, 1959, Total soluble sugar Jayaraman, 1981, Protein content Lowry, *et al.,* 1951, amino acid content, leaf nitrate Cataldo, *et al.,* 1978, *in vivo* nitrate reductase activity Jaworski, *et al.,* 1971, peroxidase and catalase activity Kar and Mishra, 1976.

RESULTS AND DISCUSSION

The physico – chemical characters, analysis were tabulated in Table 1. The morphometric characters such as root length, shoot length, leaf area, fresh weight and dry weight decreased (Fig. 1) with increasing in the concentration of Match industry effluent. Similarly, chlorophyll, carotenoids, total soluble sugar, protein and nitrate reductase activity also showed declining in trend. In contrary, the anthocyanin, leaf nitrate, free amino acid, proline contents and the activities of antioxidant enzymes such as peroxidase, catalase were increased (Fig. 2).

Match industry effluent contained 1627 mg/L of total dissolved solids and 850 mg/L of total hardness, which leads to root and shoot length inhibition at higher concentration of the effluent. The pronounced inhibition of shoot and root growth and leaf area were the main cause for the decrease in fresh and dry weight of seedlings. The inhibition of biomass accumulation is directly related to the photosynthetic process. At higher concentration, the effluent showed inhibitory effect on both photosynthetic pigments and total soluble sugar. Similar result was observed by Kumar, 1999 after the irrigations with effluent.

The reduction in sugar content maybe attributed to reduction in chlorophyll content of the leaf and also a decline in protein. This change might have already affected the photosynthetic activity of the plant and hence reduction in contents Swaminathan, *et al.,* 1998.

Accumulation of proline has been frequently used as biochemical marker for water stress in plants Alia and Saradhi, 1991. An increase in the aminoacid and proline content after match, sugar industrial effluent treated has already been reported Ramasubramanian, *et al.,* 2006. The leaf nitrate content was found to be more in effluent treated plants paralleling with the reduction in *in vivo* nitrate reductase activity.

In the present study an enhanced peroxidase activity was observed with the increase in the concentration of Match industrial effluent. peroxidase plays a vital role in IAA and chlorophyll degradation, thus observed increase in peroxidase activity can be correlated with the observed reduction in chlorophyll content, fresh weight and biomass Balasimha, 1982. Paper industrial effluent also caused similar increase in peroxidase activity of *Eleucine corocana* Selvarathi, *et al.,* 2010.

Catalase is antioxidant and scavenging enzyme, it was found to be increased with the increasing concentration of Match industry effluent. Catalase is special type of peroxidase enzyme which catalyses the degradation of H_2O_2, which is natural metabolism and also toxic to plants Jayakumar and Ramasubramanian, 2009.

Bioadsorption studies showed that the morphometric features increased (Fig. 3) by the application of seaweed powder. The chlorophyll content increased (Fig. 4) with increasing amount of seaweed powder. This result coincides with the results of Ramasubramanian, *et al.,* 2006. The total soluble sugar, protein content and nitrate reductase activity also increased (Fig. 4) after the application of seaweed powder.

Table 1. Physico-chemical analysis of Match Industry Effluent

S.N.	Parameters	BSI Standards	Value Characters
1.	Temperature	-	30.8ºC
2.	pH	7-8.5	8.2
3.	Electric Conductivity (EC)	400	1175
4.	Total Dissolved Solids (TDS)	500	1627
5.	Total Hardness	300	850
6.	Biological Oxygen Demand (BOD)	-	325
7.	Dissolved Corbandioxide	22.8	182

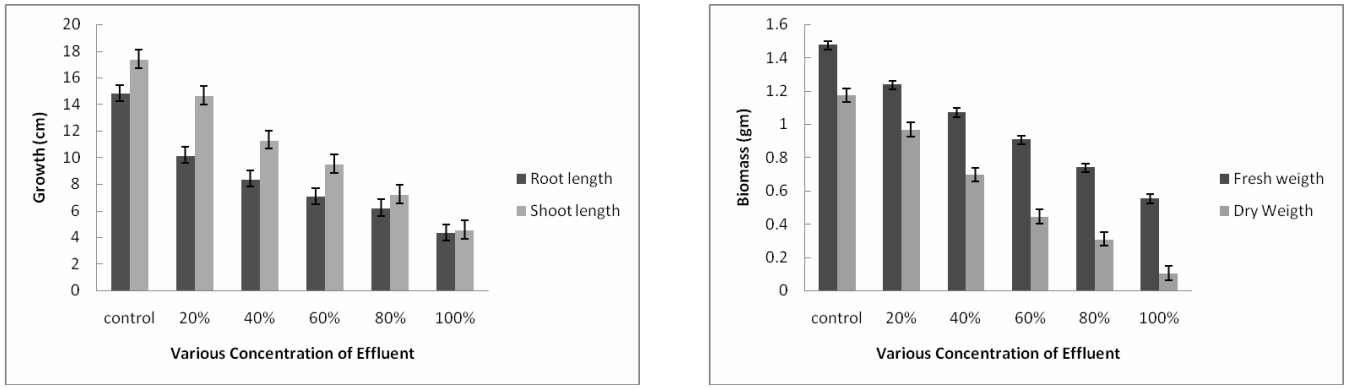

Fig. 1. Impact of various concentration of Match Effluent on the Morphometric characteristics of *Cyamopsis tetragonoloba* Taub.

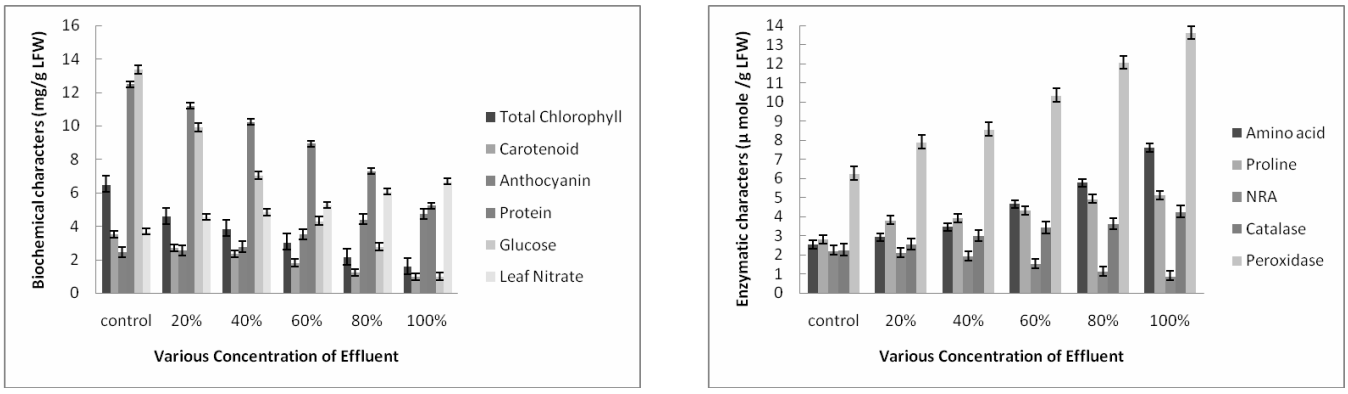

Fig. 2. Impact of various concentration of Match effluent on the photosynthetic pigments, biochemical and enzymatic characters of *Cyamopsis tetragonoloba* Taub.

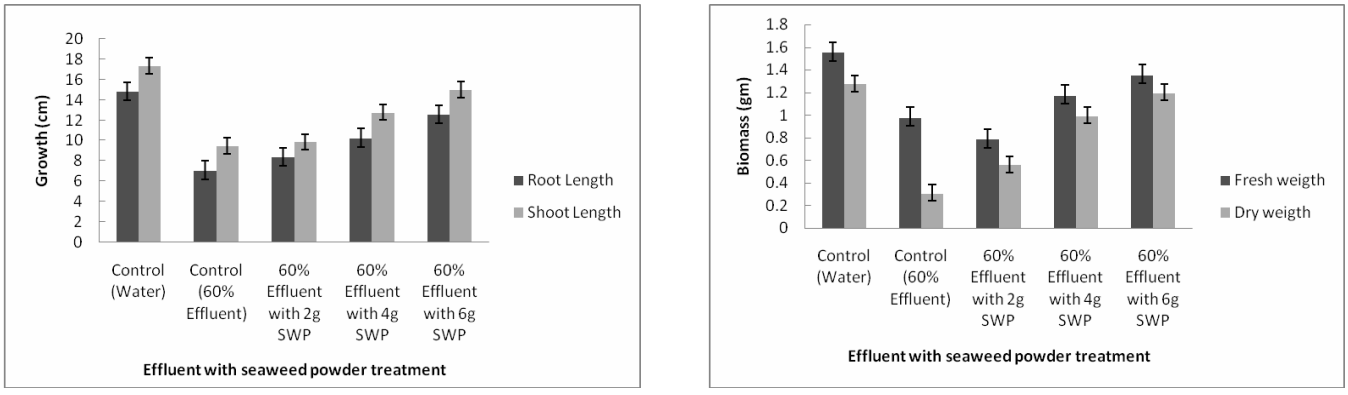

Fig. 3. Effect of Match Effluent and *Sargassum wightii* on the Morphometric characteristics of *Cyamopsis tetragonoloba* Taub.

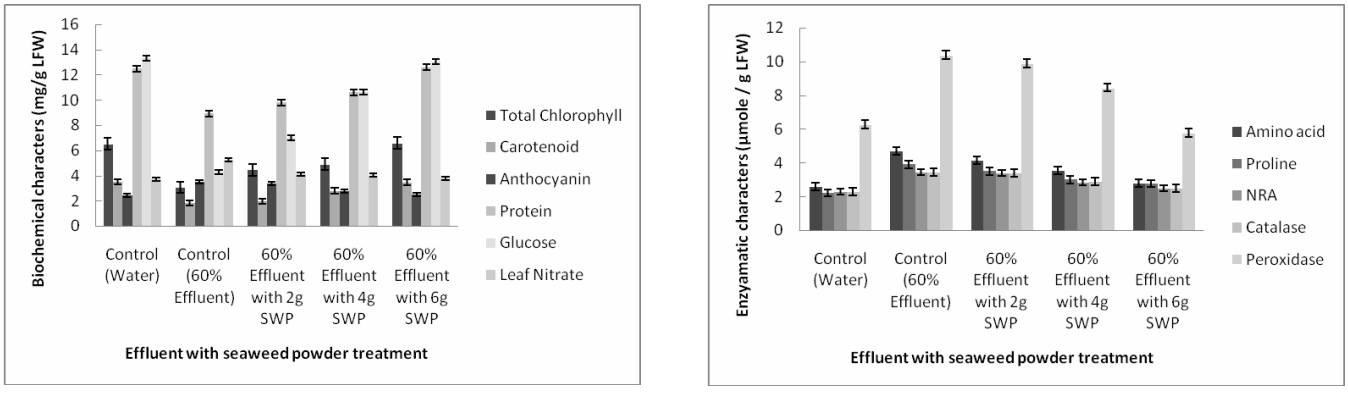

Fig. 4. Effect of Match Effluent and *Sargassum wightii* on the photosynthetic pigments, biochemical and enzymatic characters of *Cyamopsis tetragonoloba* Taub.
Abbreviations: g–Gram LFW–Leaf Fresh Weight mg–milligram NRA–Nitrate Reductase Activity SWP–Seaweed powder

This may be due to the bioadsorption by plant biomass (*Sargassum wightii* powder) to remove / adsorb toxic elements which are present in effluent Selvarathi, *et al.,* 2010. In contrary, leaf nitrate, free aminoacid and proline and the activity of enzymes such as catalase and peroxidase were found to be reduced after the application of seaweed in our studies.

Conventional methods of removal of toxins present in the Match industrial effluent are expensive and hence the use of low cost environment friendly bioadsorbants has been tested. The dried algal biomass used in the present study is available in large quantities in the East Coast area of Indian subcontinent. This can be utilized for removal of heavy metal and also found to be a potential, economic and effective safe alternative Jayakamar and Ramasubramanian, 2009.

Result of the present study clearly shows that the green algae used i.e. *Sargassum wightii* can efficiently remove the

toxicity from effluent. Hence we strongly suggest that *Sargassum wightii* can be used as a bioadsorbant to remove the toxicity of effluent polluted environment for sustainable agriculture.

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Spontaneous Rupture of Uterine Horns During Parturition- Case Report

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ABSTRACT

A 4 year old Labrador female dog was presented with the history of completing 62 days of her gestation. Obstetrical examination revealed that the cervix was dilated with the outermost fetus in anterior presentation. Celiotomy was performed through a caudal mid-ventral incision, one dead fetus was present in the peritoneal cavity through the tear at the junction of the right uterine horn and the body and five more dead featuses were found in the uterus. After removing all the fetuses, ovariohysterectomy was performed. The female dog recovered uneventfully.

Key words: *Gestation, dead fetuses, peritoneal cavity, Celiotomy, female dog*

Rupture of the gravid uterus is an unusual finding in a bitch but is occasionally seen in periparturient period in cases of dystocia (Hajurka, *et al.*, 2005). Stone, *et al.,* 1993 stated that uterine rupture in a pregnant bitch can occur following uterine torsion or trauma. It can be also caused by excessively large doses of oxytocin (Jackson, 2004)

History and Diagnosis: A 4 year old Labrador female dog was presented with the history of completing 62 days of her gestation. She began the act of parturition the previous night with the expulsion of fetal membranes but had not delivered a foetus for over 12 hours. Obstetrical examination revealed that the cervix was dilated with the outermost foetus in anterior presentation. The female dog was administered Inj. Oxytocin prior being presented to the Teaching Hospital, Veterinary College, Jabalpur. Her prior whelping was uneventful with the delivery of five healthy puppies.

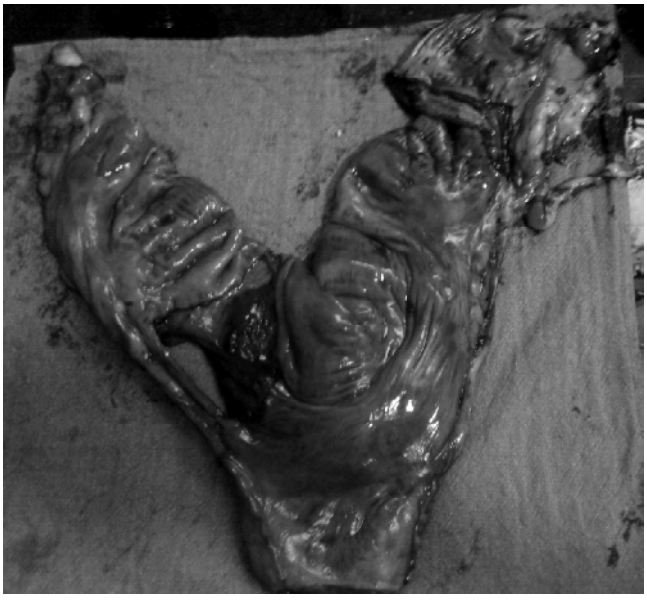

Fig. 1. The uterus of the labrador female dog after ovariohyterectomy. The uterine tear at the junction of the right uterine horn with the body and at the proximal aspect of the left uterine horn.

MATERIALS AND METHODS

Prior to the operation, prophylactic antibiotics and analgesic i.e. Inj. Amoxirum Forte-300mg i.m. and Inj. Meloxicam @ 0.5 mg/kg i.m. The female dog was anesthetized with Inj Diazepam @ 2mg/kg wt i/v; Inj ketamine @ 8mg /kg i/v. During the operation, fluid therapy was applied. Celiotomy

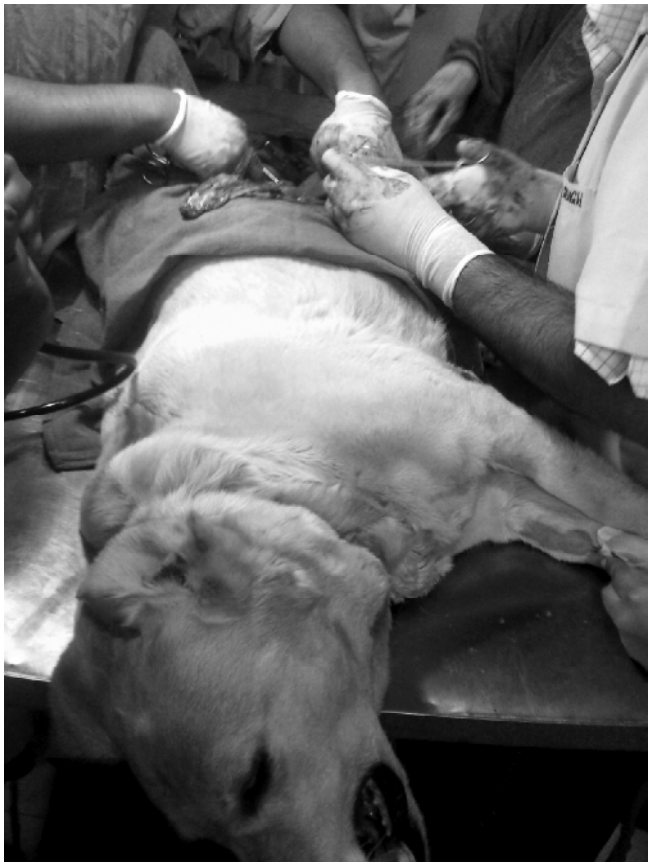

Fig. 2. During the operation

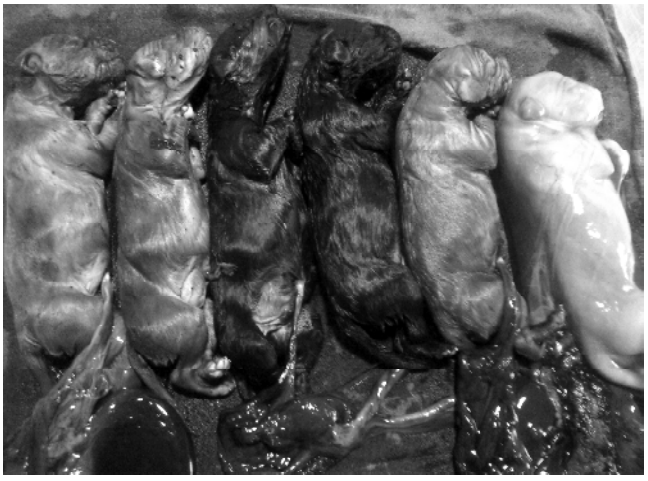

Fig. 3. The dead puppies with the fetal membranes

was performed through a caudal mid-ventral incision. A foetus was found in the peritoneal cavity through the tear at the junction of the right uterine horn and the body. Another uterine tear was identified at the proximal end of the left uterine horn. Five foetuses were found within the uterus, however all six were dead. Ovariohysterectomy was performed.

RESULTS AND DISCUSSION

Preparturient rupture of the uterus often results from external trauma. Rupture during whelping is most likely to occur in cases in which the uterine wall is compromised by the presence of infection, a dead foetus, uterine torsion, or careless obstetrical procedures. (Hajurka, *et al.*, 2005).

Johnston, *et al*., 2001 warned that ecbolic drugs should not be used if obstructive dystocia is present because uterine rupture may ensue. In this case, although Oxytocin was administered, it was ensured that the cervix was dilated and the foetus was in anterior presentation, thus the likelihood of the uterine rupture occurring due to oxytocin is minimal. The uterine rupture most likely occurred due to extensive pushing by the female dog, which went unnoticed by the owner.

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Combining Ability and Heterosis for Seed Yield and Its Component in Linseed (*Linum usitatissimum* L.)

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ABSTRACT

Combining ability and heterosis were calculated in linseed during 2010-11 following line ×tester design for 10 quantitative characters using six lines and three testers. The mean square for variation among parents and hybrids were highly significant for all the traits. The variation due to parents vs. hybrids was highly significant for all the characters except days to 50% flowering indicating significant heterotic response. Combining ability studies revealed that the parents Polf 17-1 and EC 41528 were good general combiners for seed yield/plant and most of its components, whereas A 195 (178) and A 196 (179) were good general combiners for yield components only. Based on the comparison of mean performance, SCA effects and the extent of heterosis, the hybrids A 238 (196) X EC 41528 and A 72 (112) X EC 41528 appeared to be the most promising for seed yield. In general, the hybrids excelled their respective parents and the standard checks for most of the characters studied. Significant general combining ability (GCA) and specific combining ability (SCA) effects revealed meaningful contributions of additive and non-additive type of gene actions in governing the traits. GCA/SCA found less than 1 revealed the importance of non-additive gene action in the inheritance of all the traits and stresses the need for its exploitation either through heterosis breeding programme.

Key words: *Line X Tester cross, Linseed (Flax), General combining ability, Heterosis, Specific combining ability.*

Linseed (*Linum usitatissimum* L.) is an oldest domesticated and economically important industrial nonedible oilseed crop which is being cultivated for seed and its fiber since centuries (Damania, 1997). The success of any hybridization programme chiefly depends on combining ability of parents used in crossing programme (Hallauer and Miranda, 1981). Combining ability provides an important tool for selection of desirable parents and to get required information regarding the nature of gene action controlling desirable trait (Sprague and Tatum, 1942). Kempthorne's methods of line X tester analysis have been widely used to provide reliable information on the nature and magnitude of gene effects that contribute to the expression of quantitative traits and to help plant breeders select appropriate parents for hybridization (Kempthorne, 1957). In view of the above facts and in order to develop and identify productive varieties, the present investigation were undertaken using Line x Tester analysis to derive information on the genetic parameters for various quantitative characters.

MATERIALS AND METHODS

The experimental material comprised six promising lines of linseed (having higher yield and better agronomic characters), which were crossed with three different testers (having broad genetic base and wider adaptability) in Line X Tester fashion to generate 18 F1. The seeds of 30 entries (9 parents, 18 F1 hybrids and 3 checks) were sown in the field using a randomized complete block design with three replications on 23rd November 2010. This experiment was carried out at Field Experimentation Centre of the Department of Genetics and Plant Breeding, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad (U.P.). Each plot consisted of three rows 30 cm apart and 2 m long with plant to plant distances of 7 cm. The standard agronomic practices for linseed were followed during the growing season. Days to flowering and days to maturity were recorded on plot basis, whereas plant height, primary branches per plant, number of capsules per plant, number of seeds per capsule, seed yield per plant (g), 1000-seed weight (g), and harvest index (%) were recorded using five randomly selected plants from each plot. The mean values of different traits were subjected to Analysis of Variance estimation of Heterosis heterobeltiosis economic heterosis and combining ability analysis.

RESULTS AND DISCUSSION

Flowering and maturity: The ANOVA showed significant differences among the parents and hybrids for both days to flowering and days to maturity while significant differences among parent *vs* hybrids for days to maturity (Table 1). Days to flowering among the hybrids varied from 78.67 to 88.67 days belonged to A196 (179) X EC 12082 and Polf 17-1 X EC 10077, respectively. Significant mean squares of both general and specific combining abilities revealed the importance of both additive and non-additive gene effects for these two traits; however, low GCA/SCA ratios of 0.39 for days to flowering and 0.17 for days to maturity (Table 3) indicated the predominance of non additive gene effects in their inheritance. Our findings are in agreement with those of Singh, *et al*., 2009 and Bhateria, *et al*., 2006 who reported the predominance of non-additive genetic effects for days to flowering but

inconsistent with those of Kurt and Evans, 1996 who observed a greater variance of GCA than of SCA for both days to flowering and days to maturity. The highest positive GCA effect was observed for days to flowering and days to maturity in A238 (196) parent (1.83 and 5.72 days, respectively) (Table 4). In contrast, the parent A 72(112) possessed the highest GCA effect for early flowering (-1.28 days) and early maturity (-3.5 days); thus, this parent could be used in recombination breeding for developing early maturing cultivars. The Polf 17-1 X EC 10077 hybrid had the highest and significantly positive SCA effects for days to flowering while the A 196(179) X EC 41528 hybrid showed the same SCA effects for days to maturity (Table 5). On the other hand, the highest negative and significant SCA effects of -3.33 were obtained for early flowering in the Polf 17-1 XEC 12082 cross and -5.67 for early maturity in the A 72(113) XEC 41528 cross. For early maturity, the hybrids of A 72(112) XEC 41528, A 196(179) X EC 10077 and A72(113) X EC 41528 presented significant heterobeltiosis for this. Early flowering and maturity is one of the main objectives in breeding programs for flax (Kurt and Evans, 1996) and the present findings suggest the possibility of effective genetic improvement for early flowering and early maturity in these materials.

Plant height: Genotypes including parents and their hybrids varied significantly for plant height (Table 1). Among the hybrids, A238(196) X EC 12082 with 102.4 cm and Polf 17-1 X EC 12082 with 78.2 cm had the highest and lowest plant heights, respectively. Analysis of variance for combining ability showed that GCA and SCA were significant for this trait (Table 3). These results are in agreement with the findings of Bhateria, *et al.,* 2006, but inconsistent with those of Sood, *et al*., 2007 who demonstrated that the additive effects were more important for the genetic control of plant height in linseed. Estimates for GCA effects showed that the parents A 195(178) and EC 12082 had significant and negative values (-4.49 and -3.06 cm, respectively), whereas AH92 and McGregor parents had the higher, positive and significant GCA values of 5.72 and 1.72 cm, respectively (Table 4). Both negative and positive SCA effects were observed among the hybrids for plant height (Table 5). SCA effects for plant height varied from -4.57 to 7.56 cm, belonging to A 72(113) X EC 41528 and A 196(179) X EC 41528 hybrids, respectively (Table 5).

Primary branches per plant: Analysis of variance indicated

Table 1. Analysis of variance for 10 characters in Linseed

S.No.	Characters	Mean Sum of Squares						
		Replications	Treatments	Parents	Hybrids	P v$_s$ Hy	Error	Total
		2	26	8	17	1	52	80
1	Days to 50% flowering	0.9383	29.34**	45.84**	23.30**	0.006	2.48	11.18
2	Days to maturity	3.59	96.13**	131.0**	84.83**	9.38*	1.72	32.45
3	Plant height	0.39	169.99**	201.99**	135.48**	500.72**	1.49	56.23
4	Number of primary branches/plant	0.04	29.63**	12.00**	26.97**	215.86**	0.24	9.78
5	Number of capsules/plants	28.49	31084.13**	4285.23**	26991.80**	315041.59**	207.38	10237.80
6	Number of seeds/capsule	0.38	10.64**	7.53**	10.22**	42.52**	0.27	3.64
7	1000 grain weight	0.007	3.87**	1.64**	4.57**	9.70**	0.01	1.26
8	Biological yield/plant	4.07	1108.08**	203.39**	981.98**	10489.31**	3.04	362.21
9	Harvest index	2.58	200.27**	140.54**	239.73**	7.23**	1.31	66.00
10	Seed yield/plant	0.0935	44.31**	9.0**	43.82**	335.14**	0.06**	14.44

**,*significant at 1% and 5% level of significance respectively

Table 2. Heterosis (ha), Heterobeltiosis (h_b) and Economic Heterosis (hc) for different characters in linseed

Genotypes	Plant height (cm)			Capsules per plant			Test weight (gm.)			Seed yield per plant (gm.)			Harvest index		
	Ha	Hb	HC	Ha	Hb	HC	Ha	Hb	HC	Ha	Hb	HC	Ha	Hb	HC
1. A 72(112) X EC 41528	22.56**	9.27**	27.57**	216.35**	143.27**	475.12**	-3.66**	-11.27**	-26.63**	152.48**	112.76**	221.98**	3.46	-13.24**	-18.40**
2. A 72(112) X EC 12082	-6.87**	-10.89**	4.05**	75.06**	29.62**	242.86**	8.64**	-1.71	-15.45**	114.35**	88.54**	185.32**	21.88**	-2.72	-38.04**
3. A 72(112) X EC 10077	-2.11*	-4.93**	10.99**	47.58**	21.60*	138.71**	-8.76**	-15.53**	-30.93**	10.44**	-18.75**	22.96**	-28.10**	-43.37**	-63.93**
4. A 72(113) X EC 41528	13.98**	5.62**	13.15**	-9.86	-14.51*	125.35**	4.53**	4.38**	-13.69**	31.96**	12.90**	64.68**	5.39	-16.74**	-21.69**
5. A 72(113) X EC 12082	0.00	-0.21	6.90**	136.47**	136.06**	524.42**	8.60**	6.34**	-8.52**	-25.02**	-32.98**	-2.25	-51.13**	-58.56**	-77.39**
6. A 72(113)XEC 10077	6.68**	5.28**	15.83**	15.63*	0.87	165.90**	39.10**	38.53**	14.21**	29.19**	-3.80	40.31**	77.54**	48.41**	-19.04**
7. A 195(178)XEC 41528	16.52**	15.18**	7.78**	97.75**	77.64**	427.19**	10.20**	3.76**	-14.21**	69.87**	66.93**	79.35**	13.90**	-11.38**	-16.66**
8. A 195(178)XEC 12082	9.63**	2.90*	9.78**	125.78**	113.51**	533.64**	2.08*	-5.65**	-18.83**	49.72**	44.87**	66.43**	-44.38**	-51.99**	-74.90**
9. A 195(178)XEC 10077	9.37**	1.20	11.35**	-4.67	-20.81**	135.02**	43.57**	35.88**	11.11**	77.30**	47.52**	58.50**	-35.78**	-45.39**	-71.44**
10. A 196(179)XEC 41528	21.59**	6.73**	29.15**	149.9**	132.16**	448.85**	-5.97**	-19.55**	-33.48**	12.72**	-13.15**	66.50**	-70.58**	-75.19**	-76.67**
11. A 196(179)XEC 12082	-3.98**	-9.66**	9.31**	47.34**	30.14**	244.24**	23.80**	4.21**	-10.35**	85.20**	48.10**	183.92**	50.22**	19.28**	-22.97**
12. A 196(179)XEC 10077	3.63**	-1.07	19.70**	115.24**	111.82**	329.49**	59.67**	37.24**	12.22**	66.74**	14.40**	119.31**	31.07**	2.73	-33.66**
13. Polf 17-1XEC 41528	14.70**	5.68**	14.65**	76.91**	73.27**	327.19**	9.70**	6.50**	-11.94**	224.89**	200.47**	211.66**	-23.52**	-36.64**	-40.41**
14. Polf 17-1XEC 12082	-3.34**	-4.15**	3.99**	94.23**	87.63**	396.31**	-10.71**	-14.95**	-26.83**	127.12**	100.67**	130.55**	10.16*	-11.07**	-45.04**
15. Polf 17-1XEC 10077	7.59**	6.83**	17.55**	105.83**	84.86**	355.76**	-1.65	-3.99**	-21.50**	295.16**	257.53**	215.10**	50.43**	19.82**	-25.96**
16. Polf 17-1XEC 41528	16.04**	1.38	24.02**	50.22**	38.49**	288.02**	2.20*	4.28**	7.64**	208.12**	177.12**	259.83**	45.34**	16.32**	2.35**
17. Polf 17-1XEC 12082	17.97**	10.42**	35.09**	31.13**	27.47**	257.14**	-6.81**	-7.54**	-20.46**	62.87**	53.49**	99.30**	35.82**	23.59**	-42.76**
18. Polf 17-1XEC 10077	2.61*	-2.55*	19.22**	70.21**	44.74**	305.53**	17.48**	15.47**	-2.23**	20.70**	-6.49	21.42**	-41.93**	-47.99**	-75.91**

**and*Significance at 1% and 5% level of significance respectively

Table 3. Analysis of variance for combining ability for different characters in linseed

S. N.	Characters	Mean sum of squares			
		GCA	SCA	Error	GCA/SCA
		[8]	[17]	[52]	
1	Days to 50% flowering	2.19**	5.56**	0.83	0.39
2	Days to Maturity	4.57**	26.42**	0.57	0.17
3	Plant height	13.03**	33.16**	0.49	0.39
4	Number of Primary branches/plant	1.99**	7.51**	0.08	0.26
5	Number of Capsules/plants	1971.51**	10814.89**	69.12	0.18
6	Number of Seeds/capsule	0.81**	2.51**	0.09	0.32
7	1000 grain weight	0.061**	1.05**	0.003	0.06
8	Biological yield/plant	42.91**	390.34**	1.01	0.11
9	Harvest index	9.10**	111.35**	0.43	0.08
10	Seed yield/plant	4.11**	11.41**	0.02	0.36

**,*significant at 1% and 5% level of significance respectively

that the entries effect was significant difference for primary branches per plant (Table 1). A range of 8 to 18.3 branches per plant observed for the hybrids Polf 17-1 X EC 12082 and A 196(179) XEC 12082, respectively, indicated a high variability among the hybrids. Significant mean squares of GCA and SCA (Table 3) revealed the importance of both additive and non-additive gene effects in genetic variation of primary branches per plant; however, the GCA/SCA ratio (0.26) confirmed the preponderance of non-additive genetic effects in its genetic control. These results were in agreement with earlier report Singh, *et al*., 2009 but not with those of Patil and Chopde, 1981, Sood, *et al.*, 2007 who reported a higher importance of additive gene actions on the genetic control of primary branches per plant. Estimations for combining ability effects showed positive and significant GCA values of 2.31 and 1.76 for the parents A 72(112) and A 196(179), respectively (Table 4). The SCA effects ranged from -1.98 to +4.02 branches per plant obtained for the hybrids A 238(196) X EC 41528 and A 72(113) X EC 10077, respectively (Table 5).

Seed yield and its components: Significant variations in seed yield and its components including number of capsules per plant, number of seeds per capsule, 1000- seed weight and seed yield per plant were observed among the parents and their F1 hybrids (Table 1). Analysis of variance for combining

Table 4. General combining ability effects of parents for different characters in linseed

S.N.	Genotypes	Characters									
		Days to 50% flowering	Days to maturity	Plant height	Primary/ branches per plant	Capsules/ plants	Seeds/ capsule	1000 grain weight	Biological yield/plant	Harvest index	Seed yield/plant
1	A 72(112)	-1.28	-3.50**	-1.03	2.31**	-23.33**	-0.57*	-0.88**	-3.40**	1.82**	1.42**
2	A 72(113)	-0.50	-4.39**	-2.73**	1.43**	-33.22**	1.54**	0.93**	-18.62**	2.11**	-3.77**
3	A 195(178)	1.61*	1.72**	-4.49**	-0.80**	34.33**	-1.91**	0.54**	6.13**	-3.78**	-2.16**
4	A 196(179)	-2.39**	-0.94	2.90**	1.76**	16.6*	-0.13	0.27**	5.17**	0.12	0.46**
5	Polf 17-1	0.72	1.39*	-2.65**	-2.80**	30.33**	1.65**	-0.53**	3.71**	2.99**	3.43**
6	A 238(196)	1.83*	5.72**	8.00**	-1.91**	-24.78**	-0.57*	-0.34**	7.02**	-3.25**	0.63**
7	EC 41528	-1.11*	-0.22	2.90**	0.98**	22.28**	0.43*	-0.67**	1.97**	3.56**	1.76**
8	EC 12082	-0.56	0.22	-3.06**	-0.30	35.17**	-0.63**	-0.25**	2.65**	-2.15**	-0.15
9	EC 10077	1.67**	0.00	0.16	-0.69**	-57.44**	0.20	0.92**	-4.62**	-1.41**	-1.61**

**,*significant at 1% and 5% level of significance respectively

Table 5. Specific combining ability effects for different characters in linseed

S.N.	Genotypes	Days to 50% flowering	Days to maturity	Plant height	Primary branches per plant	Capsules/ plants	Seeds/ capsule	1000 grain weight	Biological yield/ plant	Harvest index	Seed yield/ plant
1.	A 72(112)XEC 41528	-0.44	-3.89**	7.23**	1.13**	114.83**	0.24	0.48**	-2.47	4.99**	1.97**
2.	A 72(112) X EC 12082	-0.67	3.00**	-4.64**	-0.93*	-66.06**	1.30**	0.99**	4.81**	2.97**	2.13**
3.	A 72(112)XEC 10077	1.11	0.89	-2.59**	-0.20	-48.78**	-1.54**	-1.47**	-2.33	-7.96**	-4.10**
4.	A 72(113)XEC 41528	-1.89	-5.67**	-2.00*	-0.31	-128.28**	0.13	-0.25**	-9.44**	3.40**	-0.31
5.	A 72(113)XEC 12082	2.56*	6.22**	-0.77	-3.70**	147.50**	-1.48**	-0.24**	16.65**	-12.83**	-1.59**
6.	A 72(113)XEC 10077	-0.67	-0.56	2.77**	4.02**	-19.22	1.35**	0.50**	-7.21**	9.42**	1.90**
7.	A 195(178)XEC 41528	-1.33	-0.78	-4.30**	-0.43	22.50	-0.76	0.09	-33.61**	11.28**	-1.22**
8.	A 195(178)XEC 12082	2.78*	-4.89**	3.17**	1.19**	86.61**	2.63**	-0.72**	19.68**	-5.95**	0.07
9.	A 195(178)XEC 10077	-1.44	5.67**	1.14	-0.76	-109.11**	-1.87**	0.63**	13.93**	-5.32**	1.16**
10.	A 196(179)XEC 41528	0.67	7.56**	4.50**	-0.65	55.83**	-0.87*	-1.25**	26.97**	-16.26**	-4.45**
11.	A 196(179)XEC 12082	-1.89	-3.56**	-4.57**	3.96**	-105.06**	-0.81*	0.26**	-14.83**	10.60**	3.03**
12.	A 196(179)XEC 10077	1.22	-4.00**	0.08	-3.31**	49.22**	1.69**	0.99**	-12.13**	5.66**	1.43**
13.	Polf 17-1XEC 41528	0.56	4.22**	-0.94	2.24**	-45.83**	0.35	1.35**	6.36**	-4.85**	-0.53**
14.	Polf 17-1XEC 12082	-3.33**	-1.22	-3.06**	-1.81**	-8.72	-0.93*	-0.32**	-7.20**	-0.97	-2.48**
15.	Polf 17-1XEC 10077	2.78*	-3.00**	4.00**	-0.43	54.56**	0.57	-1.03**	0.84	5.82**	3.01**
16.	Polf 17-1XEC 41528	2.44	-1.44	2.08**	1.1**	45.25**	1.01**	0.33**	12.20**	1.43	4.55**
17.	Polf 17-1XEC 12082	0.56	0.44	9.87**	1.30**	-54.28**	-0.70	0.03	-19.11**	6.18**	-1.16**
18.	Polf 17-1XEC 10077	-3.00*	1.00	-5.39**	0.69	73.33**	-0.20	0.39**	6.90**	-7.61**	-3.39**

**,*significant at 1% and 5% level of significance respectively

ability showed that GCA and SCA affected seed yield and its components (Table 3), implying that additive and non-additive influenced the inheritance of these traits. The low ratio of GCA/SCA for these traits indicated (Table 4) that the role of non-additive gene effects was more important than the additive ones on the variations in these traits, a finding which agrees well with the results reported elsewhere (Bhateria, *et al.*, 2006; Kurt and Evans, 1996; Singh, *et al.*, 2009). In previous studies significant influence of both additive and non-additive genetic effects on the inheritance of seed weight and seed yield per plant was reported (Sood, *et al*., 2007) and the reported GCA variance was greater than the SCA for seed weight and seed yield per plant (Patil and Chopde, 1981). Beneficial and significant heterosis over mid-parent and better parent for number of capsules per plant was observed in some crosses with the highest heterobeltiosis of 143.2 belonging to the hybrid A 72(112) X EC 41528 (Table 5). The estimates of combining ability showed that the GCA values for number of seeds per capsule was significant and positive for the parents A202(183), A72(113) and EC41528 but negative for the parents A195(178)(Table 4). The highest SCA effect of 2.63 seeds per capsule was obtained for the hybrid A195(178) X EC 12082; however, the lowest (-1.87 seeds per capsule) was observed in the hybrid A 195(178) X EC 12082. The highest heterobeltiosis was 42.1% and belonged to the hybrid Polf 17-1 X EC 12082 (Table 2). A considerable variation was observed for 1000-seed weight among the hybrids and the highest (9.5 g) and the lowest (5.7 g) mean for this trait belonged to A72(113) X EC 10077 and A238(196) X EC 41528 respectively. The parents EC 10077, A196(179) and A202(183) had positive and significant GCA values for 1000-seed weight (Table 4). Positive and significant SCA effects were observed in some cross combinations (Table 5). Highly significant differences were observed among the F1 hybrids and the highest seed yield per plant and was ranged from 5.8 to 17.08 for the hybrids A238(196) X EC 41528 and A72(112) X EC 10077. Based on the estimates of GCA effects, the parental genotypes Polf 17-1, EC 41528, A72(112) combining ability (Table 4), indicating that they could be used as good combiners for recombinant breeding programmes. The SCA effects of the hybrids for seed yield per plant were significantly different and high SCA values of 4.55 g and 3.03 g were obtained for A 238(196) X EC 41528 and A196(179) X EC 12082, respectively (Table 5). The desirable and significant heterosis in most crosses in this study confirms the results of Shehata and Comstock, 1971. The highest heterobeltiosis of 257.5% was obtained for the cross combination Polf 17-1 X EC 10077 (Table 2). For seed yield which is the most important economic trait, there was a high variation among both the parents and their F1 hybrids. The estimate of GCA/SCA ratio for seed yield (0.36) confirmed the importance of non-additive gene actions in governing this trait.

Harvest index: The analysis of variance for combining ability showed that both GCA and SCA effects were significant for this trait (Table 3). The GCA/SCA ratio for this character was 0.08, showing the importance of non-additive gene actions in the genetic control of harvest index. The higher value of GCA effect of 3.56 and 2.99 for harvest index was obtained in parents EC 41528 and Polf 17-1 respectively (Table 4). Significant heterosis was observed in some crosses and the highest value over the better parent (48.4%) obtained in the hybrid A72(113) X EC 10077 (Table 2). Development of linseed cultivars with a high harvest index is the breeder's major purpose (Kurt and Evans, 1996) and the high genetic variation for this trait in this study indicates the possibility for its improvement in flax breeding.

It can be concluded from the present study that cross A 238(196) X EC 41528 was found best as it showed positive significant economic heterosis for seed yield per plant along with for number of capsules per plant, 1000 grain weight and biological yield per plant. The parents Polf 17-1 and EC 41528 were good general combiner for seed yield and most of the traits. Therefore, these parents either can be used to constitute a composite variety or can be used as parents for development of superior single cross hybrids. Since, these data are based on one year testing, it required further testing to confirm the consistency in the performance of hybrids over the location and/ or years to substantiate the results.

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Development of Semolina Based "*Upma*" Incorporated with Fenugreek and Chickpea Seeds Flour and Evaluation of Its Sensory and Nutritional Attributes Along with Its Glycemic Index

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ABSTRACT

To develop healthy and nutritionally rich product flour of raw, soaked and germinated seeds of fenugreek and chickpea were separately incorporated in semolina to prepare the snack product *Upma* with one control (T_0) and four treatments *viz.*, T_1, T_2, T_3 and T_4. Seeds flour of fenugreek and chickpea were incorporated at 7% (T_1), 14% (T_2), 21% (T_3) and 28% (T_4) levels in semolina. Sensory evaluation of the prepared product was done by a panel of five experts using a nine point Hedonic Scale. Assessment of nutritive values of the product was done by using the food composition tables and their analyzed values were obtained by calculation method. Results show that the highest mean score for overall acceptability was found in T_2. There was significant difference (p=0.05) in colour, texture, taste and flavour between different treatment combinations. The experimental treatments of the product were found to be richer in protein, fat, dietary fiber, iron, calcium and moisture content while they were poorer in carbohydrate and calories than control (T_0). They were also found to have lower GI values than control (T_0). From the results it was concluded that seeds flour of fenugreek and chickpea can be successfully incorporated in semolina to develop a product which can enhance the nutritional quality of the original product, can lower its GI and at the same time can enrich the variety and acceptability of diet.

Key words: *Semolina, Fenugreek, Chickpea, Glycaemic Index*

Semolina known as *Suji* and *Rava* or *Ravey* in India is the purified middling of hard wheat used in making pasta, breakfast cereals and puddings (Gisslen, 2001). Semolina is a good source of carbohydrate and protein and average source of dietary fiber. Also, it is a near high GI food (GI= 69) (Nutrition Data, 2008). Fenugreek (*Trigonella faenum graccum*) seeds are a rich source of protein (25%), lysine, tryptophan and dietary fiber (24%). They are also rich in polysaccharide galactomannan and are a good source of calcium, iron and â-carotene. They also contain certain steroidal saponins which may inhibit cholesterol absorption while fiber is thought to help lower sugar levels (Gopalan, *et al.*, 2007). It is also a very low GI food (GI = 30). Supplements of fenugreek seeds were shown to lower serum cholesterol, triglycerides and low density lipoprotein in human beings (Basch, *et al.,* 2003).

The chickpea (*Cicer arietinum*) is an edible legume. Chickpeas are an excellent source of sulfite-detoxifying molybdenum and energy-producing manganese. They are also a very good source of heart-healthy folate and a good source of muscle-building protein, digestive-supportive dietary fiber, antioxidant-promoting copper, and energy-producing phosphorus and iron. Since fenugreek seeds and chickpeas are very rich in essential amino acids, fibers, vitamins, essential trace elements and antioxidants, blending of fenugreek an chickpea seeds flour/leaves in suitable proportion with a poor source of protein, vitamins and trace elements like semolina can increase the protein and vitamins content of blends (Jood and Hooda, 2003). Due to several medicinal properties of fenugreek and chickpea the blends of fenugreek and chickpea seeds flour/leaves with common food like semolina can be a good alternative for people with certain diseases. They can be used by healthy people too for maintaining sound health and for controlling their sugar level and weight.

MATERIALS AND METHODS

The present study was carried out with appropriate methodology in the Department of Foods and Nutrition, Ethelind School of Home Science, SHIATS, Allahabad.

Flour of raw, soaked and germinated seeds of fenugreek and chickpeas were separately incorporated in semolina to prepare product *Upma* with one control (T_0) and four treatments viz. T_1, T_2, T_3 and T_4 for each product at different percent inclusion levels of fenugreek and chickpeas in semolina.

Sensory attributes of prepared products were evaluated by a panel of five experts using a nine point Hedonic Scale. Assessment of nutritive values of the products was done by using the food composition tables given in 'Nutritive Value of Indian Foods' by Gopalan and Bala, 2007 and the values obtained by the investigator were also used for the purpose. The chemical analysis of the products was done by replicating each product four times and in each replication the product was evaluated for moisture, fat, protein, iron, calcium, dietary fiber, carbohydrate and total energy. The products were also tested for their glycemic responses or Glycemic Index (GI). For determining GI values of the products a method suggested by Wolever, *et al.,* 1986 was used. The data regarding sensory attributes, chemical evaluation and GI values which were obtained during the study were analyzed statistically using different parameters.

RESULTS AND DISCUSSION

Sensory scores of different treatment combinations of *Upma* showed that T_2 (8.8) had the highest score for overall acceptability among the products containing raw seeds flour of fenugreek and chickpea followed by T_o (8.6), T_1 (8.6), T_3 (8.2) and T_4 (7.7). For *Upma* containing soaked seeds flour of fenugreek and chickpeas, the highest score belonged to T_0 (9.0), followed by T_1 (8.6), T_2 (8.8), T_3 (8.3) and T_4 (7.4) and in case of *Upma* containing germinated seeds flour T_2 (8.9) had the highest score followed by T_0 (8.3), T_1 (8.5), T_3 (8.2) and T_4 (7.7). In *Upma* containing fresh leaves of fenugreek and chickpea the highest score of overall acceptability belonged to T_2 (8.8) followed by T_0 (8.7), T_1 (8.6), T_3 (8.3) and T_4 (8.0). Significant difference among different treatment combinations was also found.

Nutrient analysis of different treatment combinations of *Upma* showed that the moisture content of *Upma* varied in the range of 27.09g/100g to 36.32g/100g. The lowest value (27.09g/100g) was found in T_0 while the highest value (36.32g/100g) belonged to the treatment T_4 (soaked seeds). The lowest value of fat (15.48g/100g) was found to be in the control (T_0) whereas the highest value (17.38g/100g) was observed in the treatment T_4 (raw seeds). The lowest value of protein (6.63g/100g) was found in T_0 while the highest value (11.23g/100g) belonged to the treatment T_4 (germinated seeds). The iron content of *Upma* was found to be in the range of 1.15mg/100g to 2.98mg/100g. The lowest value (1.15mg/100g) was found to be in the control (T_0) whereas the highest value (2.98mg/100g) was observed in the treatment T_4 (germinated seeds). The lowest value of calcium (22.00mg/100g) was found in T_0 while the highest value (89.92mg/100g) belonged to the treatment T_4 (germinated seeds). The lowest value of dietary fiber (0.32g/100g) was found to be in the control (T_0) whereas the highest value (2.73g/100g) was observed in the treatment T_4 (germinated seeds). The carbohydrate content of *Upma* varied in the range of 33.89g/100g to 43.57g/100g. The highest value (43.57g/100g) was found in T_0 while the lowest value (33.89g/100g) belonged to the treatment T_4 (germinated seeds). The total energy content of *Upma* was found to be in the range of 337.10 Kcals/100g to 338.25 Kcals/100g. The highest value (338.25 Kcals/100g) was found to be in the control (T_0) whereas the lowest value (337.10 Kcals/100g) was observed in the treatment T_4 (germinated seeds).

Mean scores for GI of different treatment combinations of *Upma* showed that T_4 (64) had the lowest GI value for the products containing Raw seeds flour of fenugreek and chickpea followed by T_3 (75), T_2 (80) and T_1 (86) clearly indicating that GI decreased as the proportion of raw seeds flour increased in the product. Similarly, for products containing Soaked seeds flour of fenugreek and chickpea, the lowest GI value belonged to T_4 (67), followed by T_3 (76), T_2

Table 1. Nutrient contents (per 100 gm) of control and treated samples of *Upma* incorporated with seeds (raw, soaked and germinated) flour of Fenugreek and Chickpea

Treatment / Nutrient	Control	Raw seeds				Soaked seeds				Germinated seeds			
	T_0	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Moisture (g)	27.09	28.26	29.44	30.61	31.79	32.85	34.02	35.14	36.32	31.42	33.92	34.04	35.81
Fat (g)	15.48	15.96	16.43	16.91	17.38	15.63	15.98	16.46	16.96	15.52	15.84	16.28	16.75
Protein (g)	6.03	7.16	8.28	9.41	10.53	7.31	8.43	9.55	10.62	7.46	8.56	9.93	11.23
Iron (mg)	1.15	1.68	1.94	2.34	2.73	1.74	2.11	2.45	2.89	1.81	2.23	2.64	2.98
Calcium (mg)	22	38.5	55.21	71.50	88.42	40.2	56.42	72.14	89.26	40.86	57.10	73.23	89.92
D. fiber (g)	0.32	0.85	1.39	1.92	2.46	0.94	1.48	1.99	2.52	1.08	1.53	2.12	2.73
Carbohydrate(g)	43.57	41.34	39.11	36.88	34.65	40.84	38.62	36.23	34.11	39.86	37.92	35.62	33.89
Energy (Kcals/100g)	338.25	338.12	337.95	337.60	337.42	337.80	337.63	337.39	337.30	337.68	337.49	337.28	337.10

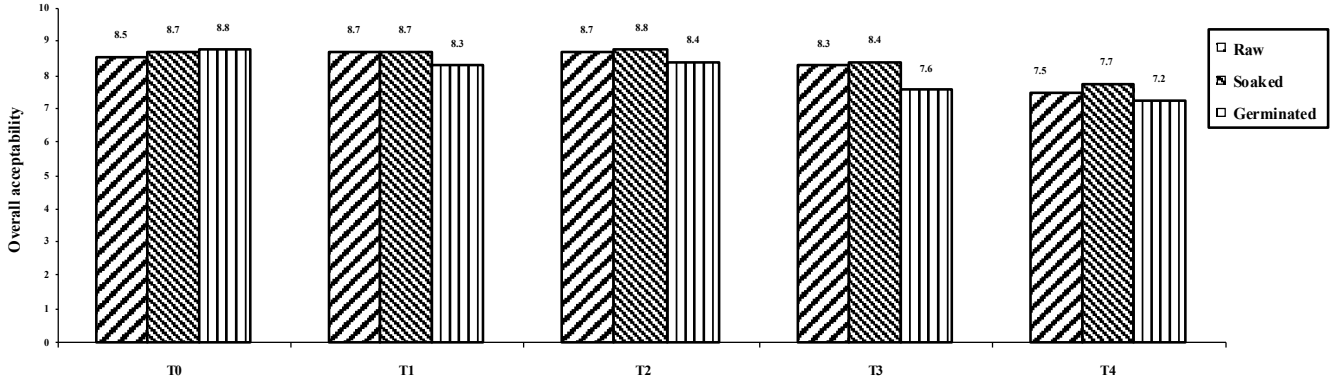

Fig. 1. Effect of Incorporation of Fenugreek and Chickpea seeds Flour (Raw, Soaked and Germinated) on the overall acceptability of *Upma*

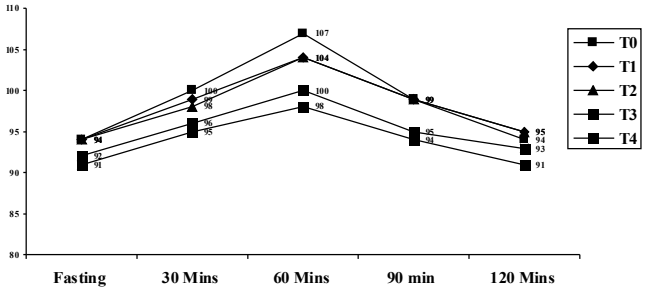

Fig. 2. Blood Glucose Response Curve for Control T_0 and Treated *Upma* Incorporated with Raw Seeds Flour of Fenugreek and Chickpea

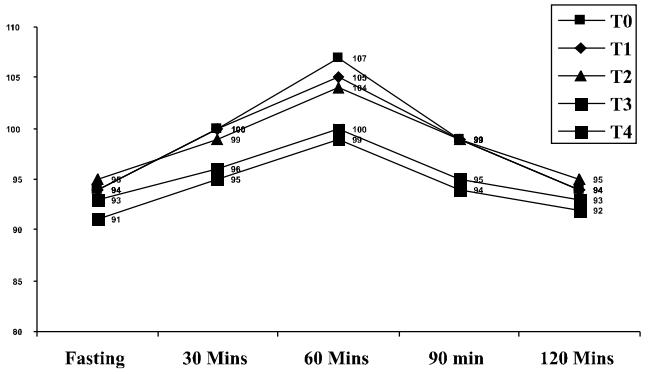

Fig. 3. Blood Glucose Response Curve for Control T_0 and Treated *Upma* Incorporated with Soaked Seeds Flour of Fenugreek and Chickpea

(83) and T_1 (88). Similar pattern had been observed for the product containing Germinated seeds flour of fenugreek and chickpeas with T_4 (63) having the lowest GI followed by T_3 (69), T_2 (78), and T_1 (83).

From the data and results it can be concluded that seeds of fenugreek and chickpea can be successfully incorporated in semolina to prepare snacks like *Upma* with an improved sensory and nutritional profile. For sensory attributes we can say that the highest score of overall acceptability in the prepared products belonged to either T_2 or T_1 which suggests that sensory characteristics of the products are improved at lower levels (upto 18%) of incorporation of fenugreek and chickpea but are deteriorated with higher levels of incorporation. This is similar to the findings reported by Jood and Hooda, 2003. Regarding the nutritional properties of the products it can be said that protein, iron, calcium and dietary fiber content of the product increases considerably with

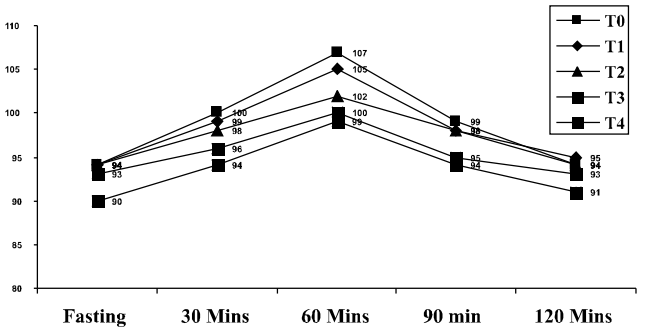

Fig. 4. Blood Glucose Response Curve for Control T_0 and Treated *Upma* Incorporated with Germinated Seeds Flour of Fenugreek and Chickpea

increase in the level of incorporation of seeds of fenugreek and chickpea in semolina while carbohydrate and total energy content of the products decreases with increase in level of incorporation. Fat and moisture content increases slightly with increase in level of incorporation. As far as the GI of the products is concerned it can be said that the GI of the products decreases substantially with increase in the level of incorporation. Particularly the products containing germinated seeds flour of chickpea and fenugreek exhibit lower GI than other products do. These results are also supported by the findings reported by Sampath Kumar, *et al.,* 2011.

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Seasonal Dynamics of Physico-chemical and Bacterial Properties of Upper Lake (Bhojtal), Bhopal, India

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ABSTRACT

Lake water ecosystem is greatly influenced by the seasonal variation in physic-chemical parameter and also due to the human interference and activities. Upper Lake (Bhojtal), Bhopal, India, is main source of drinking water to half of the population of Bhopal city, was studied to find out the seasonal variation in and correlation of physico-chemical and bacteriological parameter during 2010-12. Seasonal variation of parameter was observed with great correlation with bacterial properties. Increased turbidity, TDS, BOD and COD values indicated the high load of organic pollution and therefore the bacterial load. Catchment area of Lake under old Bhopal city was highly polluted with abiotic and biotic (TVC, TC and hemolytic bacteria) as compared to other sites of lake. Year-wise increased pollution at other sites of lake is of great concern due to urbanization and increased population, resulting into increased inflow of sewage. The increased population of total coliform and hemolytic bacteria is of great concern which is making the water unhealthy for drinking purpose before the proper treatment.

Key word: *Water Bacteria, Lake Ecosystem, water properties, Bhojtal*

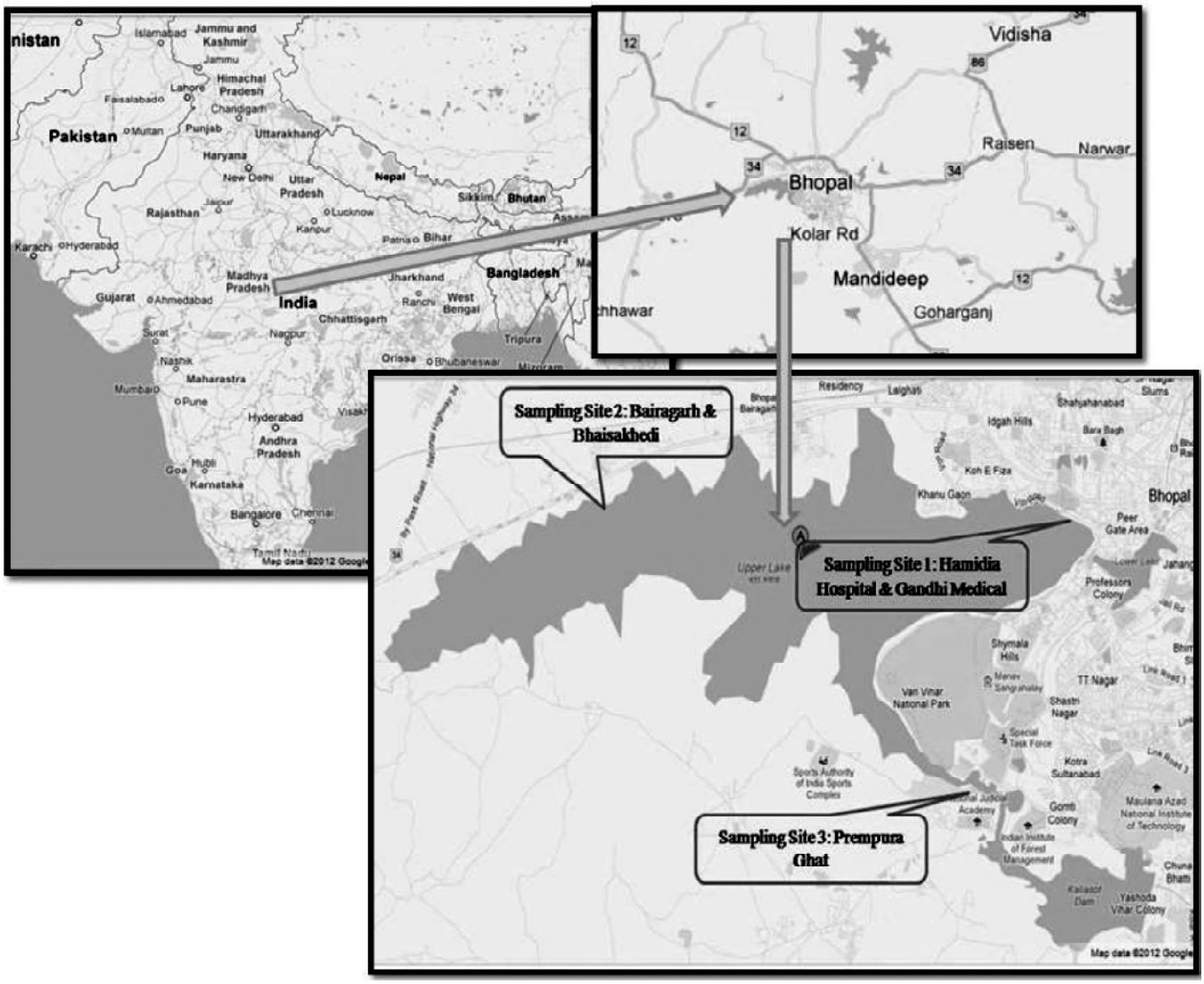
Water resources comprising of rivers, lakes, ground water, marine and costal is precious gift by nature supports all living forms on the earth. The physical and chemical parameters of water are important as it directly affects its quality and consequently its suitability for the distribution and production of fish and other aquatic animals (Toma, 2011). Microbiota composition varies as a function of water class, and depends mainly on salt and organic compound concentration, turbidity, temperature, and contamination sources (Bahgat, 2011). Large amounts of organic wastes are expected to decrease bacteria diversity (Van Es, *et al.,* 1980), but also this may select specific microbial populations to dominate in the environment (Brummer, *et al.,* 2000). Bacterial flora varies with the different regions of lake or ponds due to variable environment (Ampofo and Clerk, 2003). Municipal sewage of mixed nature and hospital effluent contains wide range of human enteric pathogens as compared to agricultural water runoff, creating health-hazard for human population (Dionisio, *et al*., 2002). In recent years, the human interference through urbanization and industrialization has placed an impact on the microbial diversity of lakes of Bhopal city. Lake water generally contains both pathogenic and non-pathogenic microbes derived from small runoff, domestic sewage, industrial effluents, agricultural activities, wild life and indigenous microorganisms. The concept of nutrients overloading and dynamic change has great impact on all subsequent eutrophication research and lake management (Vollenweider, 1976). Therefore, the physico-chemical and microbiological analysis of surface water is important towards the meaningful impact assessment of domestic and industrial activities (Ogbonna, 2010).

The upper lake was created in 11th century AD and lower lake in 18th century AD, are by far the most important. Upper Lake is heavily receiving pollutants by domestic raw sewage from surrounding habitation, agricultural and industrial waste, floral offering, immersion of idols and hospital effluents. This has lead to the reduction in storage capacity and increased pollution of lake. The changed nutrients content of the lake has adversely affected the diversity of indigenous fish, plant, animals and bacterial population. The present study was aimed with the objective to find out the correlation between the physico-chemical parameter with that of the bacterial parameter.

MATERIALS AND METHODS

Study Area: Ecologically and geographically three different sites of Bhojtal (Bhojwet land) were chosen for study purpose as given in Figure 1. Sampling site-1 was water shed area of Hospital (Hamidia and Gandhi Medical college and other hospitals) and domestic sewages from a densely populated area; sampling site-2 was under the water shed area of Bairaghar and Bhaisakhedi villages. Sampling site-3 is Prempura Ghat, a region of junction between Bhojtal and Kaliyasote dam under the watershed area of agricultural land and sewage of rarely populated area.

Sampling: Water samples at different sites were taken from Bhojtal Lake during different seasons of two years, 2010-2012, to observe the relative changes within diversity of bacterial communities. Surface layer water sample were sampled in sterile plastic bags and transferred to laboratory for further work. Physico-chemical properties of water sample were analyzed by the methods given by Golterman, *et al*., 1978 and APHA, 1995 for the parameters such as Dissolved Oxygen (DO), pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Conductivity, Alkalinity, Turbidity, Total Dissolved Solids (TDS), total hardness, Ca and Mg hardness.



(Courtesy: http://maps.google.co.in/maps)

Fig. 1. Sampling sites

Isolation of Bacteria from the water sample: The isolation of bacteria from water samples was done by the methods of Speck, 1976 and Colins and Lyne, 1985. The collected samples were serially diluted and each diluted sample was inoculated on plates (triplicates) having different media by surface spread method (Speck, 1976). Three different media as NAM (Nutrient Agar Media) for total viable count (TVC), MacConkay agar medium for Total coliform (TC) and Blood Agar Medium for Hemolytic Bacteria (HB) were used to isolate different groups of bacteria. Colony count was measured in terms of CFU (Colony Forming Unit) values.

Data analysis: Data was tabulated and processed using MS-Excel 2007. Average, standard deviation, Pearson's correlation calculations and two-way ANOVA were performed using PAST (Hammer, *et al.,* 2001) software.

RESULTS AND DISCUSSION

Physico-chemical properties

Physico-chemical characteristics of the water were investigated for the year 2010-12, to determine the level of contaminant in upper lake. The water temperature ranged from 26^0 C to 33^0 C and 26^0 C to 35^0 C during the year 2010-11 (Table 1) and 2011-12 (Table 2) respectively. Site-1 has slightly high temperature than site-2 and site-3. Significant correlation was observed ($p < 0.01$) between water temperature and bacterial population (TVC, TC and Hemolytic bacteria) (Table 3). Most of the bacteria are able to tolerate slight temperature change (Olutiola, *et al.,* 2010) and significant microbial activity in water can be observed at the temperature 15^0 C or above. pH is a concentration of H^+ ions which greatly influence the

bacterial diversity as well as bacterial load. pH ranges between 6.85 to 7.3, 7.08 to 7.68 and 8.18 to 8.85 at site-2, site-3 and site-1 respectively, during all three seasons of both the years. The increased pH value during 2011-12 than 2010-11, and at site-3 as compared to other sites indicates the increased pollution level. Site-1 has high value of conductivity as compared to site-2 and site-3 during all 3 seasons, indicating high ionic pollutants at site-1. It is also noticed that the conductivity was higher during the year 2011-12 than previous year, indicating increased load of ionic pollutants during the course of time.

During the year 2010-11, DO range was between 5.58 mg/l to 7.08 mg/l, with very low concentration. During the year 2011-12, DO value range was between 5.75 mg/l to 8.90 mg/l and higher than the year 2010-11. During both the year low DO value was observed during monsoon season and sitewise high value at site-1. Site-1 has high value of BOD due to sewage with high amount of organic matter. BOD value ranged between 1.63 mg/l at site-2 in summer to 3.65 mg/l at site-1 in monsoon. Higher value of BOD during monsoon season may be due to the cause that lake receives the organic matter, swage, agricultural waste, domestic waste etc. through surface run-off from catchment area. Tamot and Sharma, 2006 reported similar results. COD values ranged from 7.18 mg/l at site-2 during summer to 9.74 mg/l at site-1 during monsoon. High value of COD was observed during monsoon and winter. During all 3 seasons, site-1 has high value of COD in both the year. Free CO_2 was comparatively high at site-1 and it was high during winter season at all three sites. But still the free CO_2 value is lower than the standard value. Low CO_2 value may be due assimilation by aquatic autotrophs conversion to carbonic acids.

The nitrate content was observed high during summer, may be due to decreased water level, high density of phytoplankton and high rate of organic decomposition. The low value of nitrate during winter may be due to low temperature, which decreases the rate of decomposition and water circulation (Tamot and Sharma, 2006). During both the year, the alkalinity was high during summer due to decreased water level. It was also comparatively high at site-1 than site-2 and site-3. Level of alkalinity was increased during 2011-12 than 2010-11 indicating increased pollution level by carbonate and bicarbonate content. The acceptable turbidity of lake water is <50 NTU (Friedman, *et al.*, 1992). The series of turbidity induced changes that can occur in a water body may change the composition of an aquatic community (Wilber, 1983).

Table 1. Physico-chemical and Bacterial Properties of Bhojtal (Upper Lake) at three different sites during three seasons (summer, monsoon and winter) of the year 2010-2011

Year 2010-11	Summer			Monsoon			Winter		
	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3
Parameter	Avg - SD	Avg -SD	Avg -SD	Avg -SD	Avg -SD	Avg –SD	Avg -SD	Avg -SD	Avg -SD
Air Temp. (^{0}C)	32.95 ± 3.66	28.70 ±1.01	29.50 ±1.29	28.46 ±0.80	26.58 ±0.43	26.43 ±0.43	27.03 ±0.75	26.08 ±0.57	26.50 ±0.48
Water Temp. (^{0}C)	30.13 ±2.02	28.25 ±1.17	28.75 ±1.04	27.06 ±0.65	25.93 ±0.48	26.03 ±0.56	25.80 ±0.91	25.80 ±0.59	25.95 ±0.34
Ph	8.18 ± 0.46	6.85 ±0.19	7.08 ±0.25	8.75 ±0.76	7.13 ±0.25	7.33 ±0.28	8.53 ±0.50	7.30 ±0.18	7.68 ±0.22
Turbidity (NTU)	10.60 ±0.45	10.18 ±0.24	10.03 ±0.17	11.85 ±1.01	10.28 ±0.36	10.78 ±0.85	9.43 ±0.43	8.05 ±0.66	8.53 ±0.68
Conductivity (µS/cm)	272.50 ±34.03	231.25 ±34.25	258.50 ±22.88	269.5 ±38.52	260.50 ±36.57	257.75 ±43.67	217.88 ±33.64	198.00 ±21.80	210.75 ±25.86
TDS (mg/l)	187.50 ±14.43	171.00 ±18.65	176.75 ±17.44	139.5 ±21.81	130.60 ±20.99	133.70 ±17.74	125.00 ±10.60	110.53 ±7.63	117.30 ±6.46
Free CO2 (mg/l)	0.90 ±0.18	0.65 ±0.13	0.65 ±0.13	1.13 ±0.22	0.98 ±0.17	0.98 ±0.17	1.33 ±0.54	0.95 ±0.13	0.95 ±0.13
D.O (mg/l)	7.08 ±0.42	6.60 ±0.47	6.63 ±0.35	6.2 ±0.50	5.58 ±0.40	5.65 ±0.39	6.88 ±0.22	6.33 ±0.26	6.40 ±0.37
B.O.D. (mg/l)	3.05 ±0.46	2.38 ±0.39	2.65 ±0.44	2.72 ±0.76	1.90 ±0.26	2.08 ±0.25	2.60 ±0.37	2.40 ±0.32	2.43 ±0.40
C.O.D. (mg/l)	9.48 ±2.23	7.18 ±0.93	7.25 ±0.91	9.75 ±2.19	8.03 ±1.00	8.08 ±1.07	9.48 ±2.16	8.00 ±0.79	8.03 ±0.74
Total Alkalinity (mg/l)	144.93 ±41.53	106.38 ±13.70	106.85 ±13.94	81.88 ±5.94	77.65 ±10.33	74.75 ±6.30	89.70 ±8.58	83.75 ±10.86	84.33 ±11.01
Total Hardness (mg/l)	132.75 ±16.28	115.23 ±10.33	117.00 ±8.45	98.93 ±4.70	93.20 ±2.44	93.05 ±3.18	108.53 ±9.51	98.55 ±5.08	100.05 ±6.04
Ca Hardness (mg/l)	24.48 ±3.21	20.03 ±1.89	19.80 ±1.68	28.78 ±3.22	25.53 ±3.77	25.25 ±3.50	28.93 ±1.12	27.73 ±1.08	27.83 ±0.87
Mg Hardness (mg/l)	18.83 ±2.15	15.43 ±2.70	14.25 ±1.51	23.65 ±1.47	16.80 ±0.81	16.95 ±0.66	20.58 ±3.12	15.80 ±0.91	16.38 ±1.01
Chloride (mg/l)	24.05 ±1.75	14.30 ±1.09	14.33 ±0.70	29.08 ±2.41	16.58 ±1.31	16.58 ±0.64	25.68 ±2.19	16.20 ±1.18	16.00 ±1.05
Nitrate (mg/l)	0.39 ±0.04	0.28 ±0.02	0.29 ±0.02	0.28 ±0.11	0.21 ±0.06	0.21 ±0.06	0.16 ±0.02	0.15 ±0.01	0.15 ±0.01
TVC (10^5 * CFU/ml)	58.07 ±9.51	38.60 ±4.87	42.93 ±0.77	43.07 ±9.03	25.33 ±4.73	25.80 ±5.66	48.07 ±6.92	31.87 ±4.28	34.00 ±4.47
TC (10^5 * CFU/ml)	28.93 ±3.52	16.6 ±3.14	19.13 ±2.72	20.2 ±3.72	11.73 ±3.2	13.07 ±2.8	21.53 ±3.14	14.6 ±2.02	15.6 ±1.71
HB (10^5 * CFU/ml)	20.07 ±3.63	13.80 ±1.76	12.47 ±1.07	14.33 ±0.91	10.60 ±1.14	8.87 ±0.90	13.47 ±1.37	10.27 ±0.86	8.47 ±1.04

TVC-TotL Viable Count, TC – Total Coliform, HB-Hemolytic Bacteria
Avg,: Average SD: Standard Deviation
Sampling Sites:
Site-1: Gandhi Medical /Hamidia Hospital
Site-2: Bhaisakhedi/Bairagarh
Site-3: Prempura Ghat
Summer season: March, April, May, June
Monsoon season: July, August, September, October
Winter season: November, December, January, February

Turbidity has low value at site-2 as compared to other sites during monsoon season (2011-12) due to rain water run-off. During dry season the high value of turbidity at site-1 indicates the particulate pollution. TDS is generally made up of inorganic salts and small amount of organic materials. Site-1 has high TDS value during all 3 season of both the years. TDS value was higher during the summer than monsoon and winter. Increased TDS value was observed during year 2011-12 than 2010-11. Total hardness was high during summer season of both the year at site-1. During monsoon and winter seasons values of hardness were significantly less varying at all 3 sites. Calcium and Magnesium values were higher at site-1 and site-3 during all season of study period. Site-1 and site-3 had shown high concentration of chloride than site-2 during 2011-12, whereas site-1 had shown concentration of chloride than site-2 and site-3 in 2010-11.

Bacterial Population Dynamics

Table 4 shows the average and standard deviation values of total bacterial count, total coliform and hemolytic bacterial count at three sites during three seasons of year 2010-12. Site-1 has high bacterial population (TVC, TC, hemolytic bacteria) as compared to site-2 and site-3. The Total Viable Count, Total coliform count and Hemolytic bacterial count were high during summer season as compared to winter, which is slightly higher than monsoon (Figure 2A, 2B, 2C). High variation of bacterial count (TVC, TC and hemolytic bacteria) was observed in various samples collected from different location of sites during summer season. The low value of bacterial load during monsoon season may be due to rain water pouring in lake, decreasing per ml bacterial count and decreased per ml organic matter of the reservoir (Table 1). Total viable count, total coliform and hemolytic bacterial counts were comparatively higher in 2011-12 than 2010-11 (Figure 2A, 2B, 2C). Marginal increase in count at site-3 (Prempura Ghat) and high increase in count at site-1 (Hamidia and Gandhi hospital) and site-2 (sub-urban area Bairaghar and village Bhaisakhedi) was observed. This may be due to dense population and increased domestic and hospital effluent at site-1 and increased population and urban resulted into the human activities at site-3. The ratio of total coliform and hemolytic bacteria to total viable count was increased during the year 2011-12 to that of the year 2010-11. This increase is prominent at the site-2 (sun-urban area Bairagarh and village Bhaisakhedi). Site-1 and site-2 has comparatively high ratio of coliform and hemolytic bacteria than site-3. Site-2 is covered with shallow water comprised of macrophytes and receives the sewage influents from domestic sewage and hospital effluents, a result of urbanization. The total coliform counts are always higher, since total coliform can originate from non-fecal source such as plants and soils (Goyal, *et al.*, 1977). Site-1 receives the domestic sewage plus the low percent hospital effluents.

Table 2. Physico-chemical and bacterial properties of Bhojtal (Upper Lake) at three different stations during three seasons (summer, monsoon and winter) of the year 2011-2012

Year 2011-12	Summer			Monsoon			Winter		
	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3	Site-1	Site-2	Site-3
Parameter	Avg SD	Avg SD	Avg SD	Avg SD	Avg SD	Avg SD	Avg SD	Avg SD	Avg SD
Air Temp. (^{0}C)	34.43 ±4.11	31.75 ±3.30	33.60 ±4.32	28.85 ±0.82	27.70 ±0.74	28.50 ±1.19	27.65 ±0.44	26.45 ±0.38	26.83 ±0.69
Water Temp. (^{0}C)	32.00 ±3.08	31.20 ±3.07	32.80 ±4.12	27.40 ±0.97	27.00 ±0.91	28.08 ±1.06	26.75 ±0.62	25.95 ±0.34	26.38 ±0.56
Ph	8.95 ±0.42	7.08 ±0.25	8.30 ±0.48	9.55 ±1.08	7.33 ±0.28	8.95 ±0.75	9.45 ±0.60	7.68 ±0.22	9.13 ±0.79
Turbidity (NTU)	11.68 ±0.30	10.03 ±0.17	10.83 ±0.48	13.10 ±0.54	10.78 ±0.85	12.20 ±0.71	10.13 ±0.30	8.53 ±0.68	9.58 ±0.43
Conductivity (µS/cm)	292.00 ±33.51	260.50 ±22.10	285.00 ±28.40	281.75 ±35.29	259.00 ±44.52	278.50 ±27.44	231.40 ±35.68	161.75 ±33.20	223.25 ±28.18
TDS (mg/l)	212.50 ±17.08	178.28 ±18.03	206.00 ±19.25	154.60 ±20.97	135.95 ±18.61	150.40 ±21.72	139.68 ±13.79	119.78 ±6.57	132.50 ±8.95
Free CO2 (mg/l)	1.30 ±0.56	0.68 ±0.10	1.20 ±0.56	1.68 ±0.51	1.18 ±0.39	1.73 ±0.22	2.43 ±0.89	1.08 ±0.28	2.05 ±0.81
D.O (mg/l)	8.90 ±0.53	6.75 ±0.34	8.50 ±0.48	7.73 ±0.22	5.75 ±0.34	7.35 ±0.34	8.20 ±0.41	6.55 ±0.30	7.83 ±0.54
B.O.D (mg/l)	2.70 ±0.42	1.63 ±0.31	2.33 ±0.46	3.65 ±0.50	2.23 ±0.35	3.18 ±0.45	3.50 ±0.26	2.55 ±0.25	3.13 ±0.10
C.O.D (mg/l)	10.63 ±2.10	7.35 ±0.91	10.20 ±1.97	11.50 ±1.82	8.25 ±1.02	11.35 ±2.12	10.78 ±2.29	8.13 ±0.73	10.13 ±2.01
Total Alkalinity (mg/l)	163.10 ±40.87	107.40 ±14.12	159.18 ±40.84	91.48 ±7.78	75.15 ±6.04	87.05 ±6.44	97.45 ±10.44	84.33 ±11.01	91.75 ±9.40
Total Hardness (mg/l)	147.23 ±19.09	141.00 ±47.78	138.90 ±14.78	104.60 ±5.72	94.55 ±3.43	97.45 ±5.50	120.28 ±9.23	100.05 ±6.04	117.13 ±10.69
Ca Hardness (mg/l)	26.75 ±2.39	20.00 ±1.68	24.70 ±3.65	30.30 ±3.12	25.55 ±3.55	29.03 ±3.33	30.75 ±1.54	27.83 ±0.87	29.98 ±1.30
Mg Hardness (mg/l)	24.95 ±2.05	14.45 ±1.53	24.25 ±1.67	28.30 ±0.93	17.10 ±0.53	27.60 ±0.73	26.60 ±2.49	16.38 ±1.01	26.15 ±2.56
Chloride (mg/l)	26.10 ±1.55	14.63 ±0.69	25.60 ±1.55	31.15 ±2.74	16.75 ±0.72	30.61 ±2.49	29.55 ±1.99	16.13 ±0.93	28.85 ±1.82
Nitrate (mg/l)	0.42 ±0.06	0.29 ±0.02	0.41 ±0.06	0.31 ±0.13	0.21 ±0.06	0.31 ±0.12	0.18 ±0.01	39.86 ±79.43	0.17 ±0.01
TVC (10^5 * CFU/ml)	65.87 ±11.50	38.33 ±11.47	47.27 ±7.77	43.20 ±7.46	27.07 ±8.07	29.53 ±5.91	47.67 ±3.92	32.93 ±8.39	33.73 ±5.81
TC (10^5 * CFU/ml)	31.87 ±4.42	17.40 ±4.47	22.87 ±3.55	22.80 ±4.13	14.73 ±3.36	16.20 ±3.40	21.67 ±1.11	15.87 ±1.19	16.60 ±3.35
HB (10^5 * CFU/ml)	26.53 ±4.43	18.00 ±2.22	14.80 ±1.59	18.53 ±1.97	12.80 ±0.90	11.27 ±0.72	15.20 ±1.54	11.73 ±0.60	10.53 ±0.51

TVC-TotL Viable Count, TC – Total Coliform, HB-Hemolytic Bacteria Avg,: Average SD: Standard Deviation

Sampling Sites:
Site-1: Gandhi Medical /Hamidia Hospital
Site-2: Bhaisakhedi/Bairagarh
Site-3: Prempura Ghat

Summer season: March, April, May, June
Monsoon season: July, August, September, October
Winter season: November, December, January, February

Correlation of Environmental variables and bacterial population

The correlation among the environmental variables and bacterial population is presented in Table 3. Correlation showed highly significant ($P<0.01$) correlation of environmental factor as air and water temperature, TDS, alkalinity, total hardness and nitrate content with the bacterial count (TVC, TC and hemolytic bacteria) at all three sites, during all three seasons of both the years. Turbidity has shown positive correlation with TC at $P<0.01$ and with TVC at $P<0.05$. It has indicated positive correlation with hemolytic bacteria. Negligible correlation value was observed between DO and bacterial count. The positive correlation between DO and BOD indicate the active propagation of autotrophs and besides the heterotrophs. This may be due to shallow water; therefore the light can reach the bottom of water, water cycle of lake, abundance of photosynthetic organisms.

Principle Component Analysis (PCA)

The result from correlation derived Principle Component Analysis of environmental factors and bacterial population within all sites during three seasons of both the years were observed and is shown in figure 3. The Component-1 (Axis-X) explained 51.57% of the total variability and showed high

Table 3. Correlation matrix of water quality parameters with the bacterial populations

	Air-Temp	Water-Temp	Ph	Free-CO2	DO	BOD	COD	Conductivity	Total-Alkalinity	TDS	Turbidity	Total-Hardness	Ca-Hardness	Mg-Hardness	Chloride	Nitrate	TVC	TC	Hemolytic
Air-Temp	1	0.00	0.51	0.54	0.92	0.01	0.29	0.00	0.00	0.00	0.07	0.00	0.12	0.42	0.39	0.00	0.00	0.00	0.00
Water-Temp	0.97	1	0.80	0.44	0.93	0.03	0.48	0.00	0.00	0.00	0.12	0.00	0.05	0.57	0.63	0.00	0.00	0.00	0.00
Ph	0.16	0.06	1	0.00	0.00	0.00	0.00	0.31	0.44	0.72	0.04	0.47	0.00	0.00	0.00	0.43	0.20	0.19	0.29
Free-CO2	-0.16	-0.19	0.85	1	0.00	0.01	0.00	0.93	0.74	0.49	0.30	0.95	0.00	0.00	0.00	0.55	0.98	0.97	0.99
DO	0.02	-0.02	0.90	0.87	1	0.00	0.00	0.21	0.93	0.98	0.01	0.88	0.00	0.00	0.00	0.59	0.89	0.50	1.00
BOD	0.57	0.51	0.84	0.62	0.71	1	0.00	0.11	0.01	0.02	0.04	0.01	0.12	0.00	0.00	0.02	0.00	0.00	0.01
COD	0.27	0.18	0.96	0.80	0.94	0.83	1	0.09	0.28	0.43	0.01	0.48	0.00	0.00	0.00	0.15	0.23	0.14	0.31
Conductivity	0.67	0.63	0.25	0.02	0.31	0.39	0.41	1	0.05	0.00	0.00	0.13	0.45	0.10	0.12	0.00	0.05	0.01	0.10
Total-Alkalinity	0.93	0.89	0.19	-0.08	0.02	0.60	0.27	0.47	1	0.00	0.38	0.00	0.24	0.40	0.40	0.00	0.00	0.00	0.00
TDS	0.96	0.96	0.09	-0.18	-0.01	0.53	0.20	0.70	0.90	1	0.04	0.00	0.03	0.49	0.55	0.00	0.00	0.00	0.00
Turbidity	0.43	0.38	0.49	0.26	0.58	0.50	0.62	0.85	0.22	0.48	1	0.66	0.67	0.01	0.01	0.00	0.23	0.02	0.35
Total-Hardness	0.87	0.87	0.18	-0.01	-0.04	0.59	0.18	0.37	0.90	0.84	0.11	1	0.16	0.53	0.51	0.00	0.00	0.00	0.00
Ca-Hardness	-0.38	-0.47	0.77	0.79	0.78	0.38	0.71	-0.19	-0.29	-0.50	0.11	-0.35	1	0.00	0.00	0.16	0.35	0.45	0.30
Mg-Hardness	0.20	0.14	0.95	0.84	0.95	0.82	0.98	0.40	0.21	0.17	0.62	0.16	0.70	1	0.00	0.22	0.33	0.24	0.46
Chloride	0.22	0.12	0.96	0.79	0.87	0.79	0.97	0.38	0.21	0.15	0.59	0.17	0.70	0.97	1	0.23	0.23	0.22	0.35
Nitrate	0.92	0.89	0.20	-0.15	0.14	0.55	0.35	0.82	0.82	0.93	0.67	0.67	-0.34	0.30	0.30	1	0.00	0.00	0.00
TVC	0.83	0.74	0.31	0.01	0.04	0.63	0.30	0.46	0.86	0.82	0.30	0.85	-0.23	0.25	0.30	0.72	1	0.00	0.00
TC	0.86	0.77	0.32	0.01	0.17	0.64	0.36	0.59	0.77	0.82	0.53	0.78	-0.19	0.29	0.31	0.79	0.85	1	0.00
HB	0.85	0.76	0.27	0.00	0.00	0.62	0.26	0.40	0.87	0.81	0.23	0.89	-0.26	0.19	0.23	0.70	0.97	0.88	1

*- Significance at $p<0.01$; **- Significance at $p<0.05$

Table 4. Average CFU values with Standard deviation of Total Viable count (TVC), Total Coliform (TC) and Total Hemolytic Bacteria (HB) at three sites, during the year 2010-12.

		Year 2010-11 (10^5x CFU/ml) (Avg., SD)			Year 2011-12 (10^5x CFU/ml) (Avg., SD)		
Season	**Sites**	**TVC**	**TC**	**HB**	**TVC**	**TC**	**HB**
Summer	Site-1	58.07 ±9.51	28.93 ±3.52	20.07 ±3.63	65.87 ±11.50	31.87 ±4.42	26.53 ±4.43
	Site-2	43.07 ±9.03	20.2 ±3.72	14.33 ±0.91	43.20 ±7.46	22.80 ±4.13	18.53 ±1.97
	Site-3	48.07 ±6.92	21.53 ±3.14	13.47 ±1.37	47.67 ±3.92	21.67 ±1.11	15.20 ±1.54
Monsoon	Site-1	38.60 ±4.87	16.6 ±3.14	13.80 ±1.76	38.33 ±11.47	17.40 ±4.47	18.00 ±2.22
	Site-2	25.33 ±4.73	11.73 ±3.2	10.60 ±1.14	27.07 ±8.07	14.73 ±3.36	12.80 ±0.90
	Site-3	31.87 ±4.28	14.6 ±2.02	10.27 ±0.86	32.93 ±8.39	15.87 ±1.19	11.73 ±0.60
Winter	Site-1	42.93 ±0.77	19.13 ±2.72	12.47 ±1.07	47.27 ±7.77	22.87 ±3.55	14.80 ±1.59
	Site-2	25.80 ±5.66	13.07 ±2.8	8.87 ±0.90	29.53 ±5.91	16.20 ±3.40	11.27 ±0.72
	Site-3	34.00 ±4.47	15.6 ±1.71	8.47 ±1.04	33.73 ±5.81	16.60 ±3.35	10.53 ±0.51

Site-1: Hamidia & Gandhi Hospital; Site-2: Sub-urban area Bairagarh & Village Bhaiskhedi; Site-3: Prempura ghat

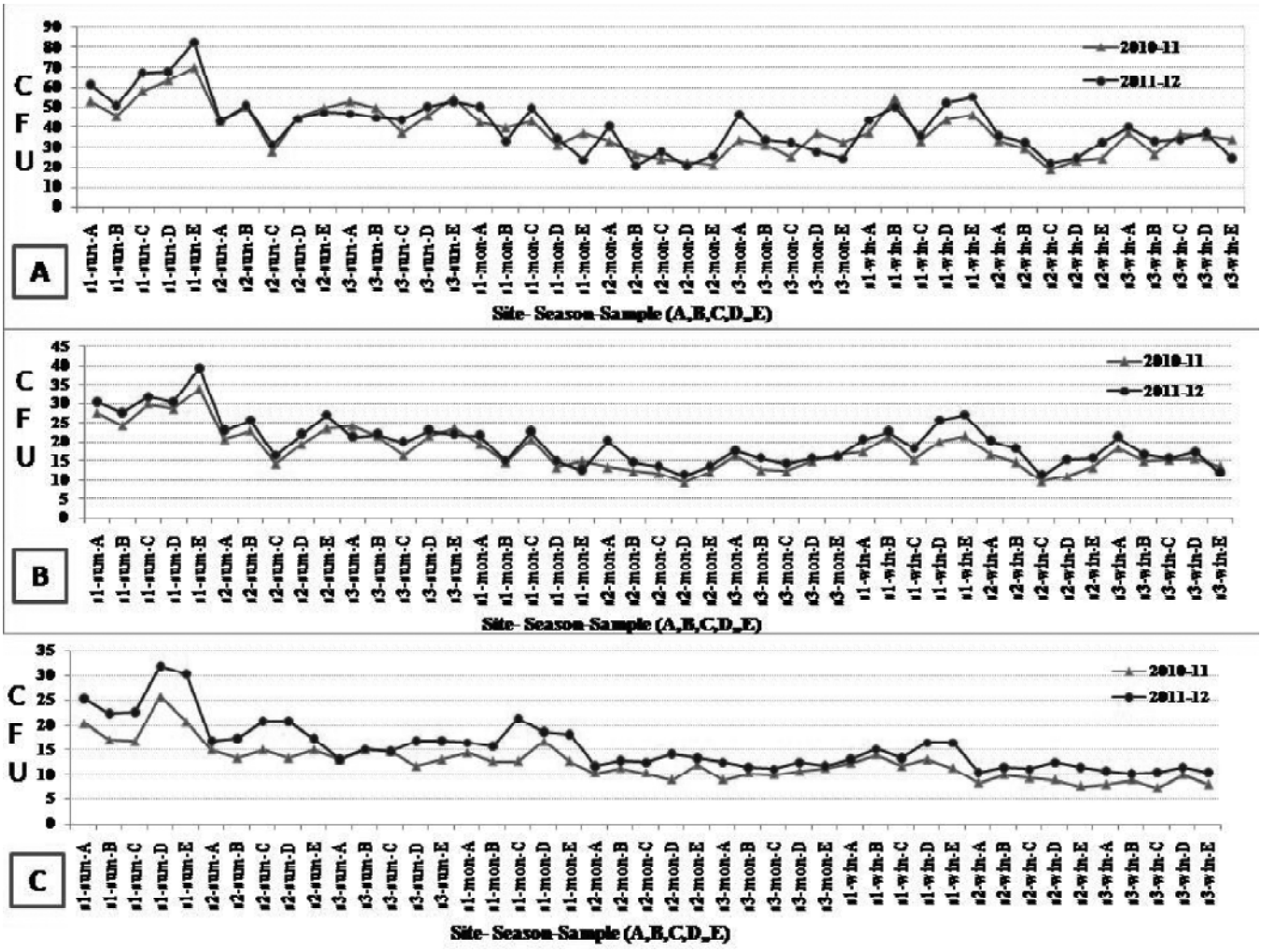


Fig. 2. Variation in bacterial population at different sites during 2010-12. (A) Total Viable Count (TVC) (B) Total Coliform (TC), (C) Hemolytic Bacteria (HB). (Site-1: s1, site-2: s2, site-3:s3; season: sum: summer, mon: monsoon, win: winter; Samples: A, B, C, D, E)

positive correlation with air and water temperature, alkalinity, conductivity, BOD, COD, TDS, Turbidity, Nitrate content and bacterial population (TVC, TC, Hemolytic bacteria) and low positive correlation with total hardness, Ca and Mg hardness, chloride, free CO_2, DO during all 3 season of both the year at site-1, summer and monsoon season of 2011-12 at site-3 and summer season of 2011-12 at site-2. All these parameters have shown negative correlation at site-2 and site-3 during winter and monsoon season of both the years. The Component-2 (Axis-Y) explained 33.26% of the variability and indicated positive correlation with total hardness, Ca and Mg hardness, chloride, free CO_2, BOD, Turbidity, COD, DO within the site-1 during monsoon and winter season of both the years and site-3 has only in year 2011-12. These parameters have shown negative correlation during other combination of season with year and sites. Conductivity, air & water temperature, alkalinity, nitrate content, TDS and bacterial population (TVC, TC, and hemolytic bacteria) have shown negative correlation with the other remaining factors but positive correlation with site-1, site-3 and site-2 during summer season of both the years.

Bhopal is developing city and speedy urbanization is in advance, all around the catchment area of Bhojtal (Upper Lake). Geographically the selected 3 sites are 3-4 km away from each other. Lake receiving sewage at site-1 from densely populated old Bhopal city inclusive of Hamidia and Gandhi medical colleges was observed to be highly polluted with biotic and abiotic factors. The catchment area of site-2 catchment area of (Biaragarh and Bhaisakhedi village) and site-3 (Prempura ghat) showed comparatively less pollution as these are under the progress of urbanization. Increased BOD and COD, besides the increased population of bacterial count indicate the increased biodegradable organic matter. Consistent increased values of physic-chemical parameter were observed during 2010-12 than in 2009 studied by Dar, *et al.*, indicating increased pollution level. The seasonal variations were observed in physic-chemical and biological

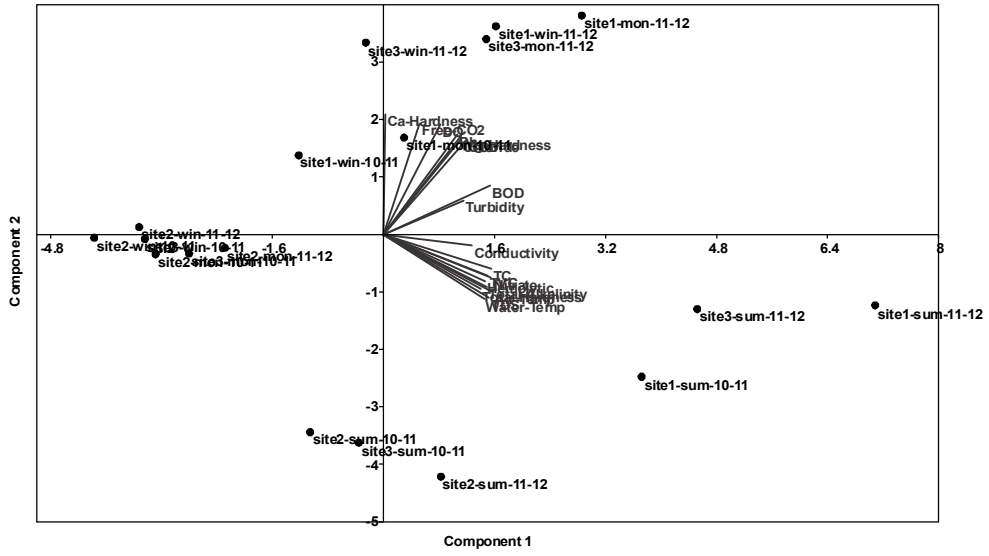


Fig. 3. Principle Component Analysis (PCA)

properties of water. The values were high in summer due to the low level of water content of reservoir and low values during monsoon may be due receive of rain runoff water. The rise of pollution level of upper lake is clearly due to the rapid growth of residential, commercial activities and other anthropogenic activities around the study area. Major concern is required in monitoring of lake and adaptation of suitable remedial measures to control the pollution and prevent the depletion of the lake water quality.

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Variability, Correlation and Path Analysis Studies in Sorghum (*Sorghum bicolor* (L.) Moench)

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ABSTRACT

Significant variations were recorded among the genotypes for various morphological and yield attributing characters in sorghum. High values for phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was recorded for grain yield/ plant (g) and fodder yield/ plant. High heritability coupled with high genetic advance was observed for plant height at maturity and fodder yield / plant and grain yield/ plant. Fodder yield /plant and 1000 grain weight were found to be significant and positively correlated with Grain yield/ plant. The result of path coefficient analysis showed that days to 50% flowering, plant height, length of panicle, width of panicle, fodder yield / plant and 1000 grain weight had positive direct effect on grain yield/ plant.

Key words: *Sarghum, Gcv, Pcv, Path coefficient analysis*

Sorghum is not only an important food grain crop but also an important fodder crop of the country. Sorghum plants are drought tolerant and can withstand water logging and soil salinity as well. Genetic improvement for quantitative traits depends on the nature, and amount of variability present in the genetic stock and the extent to which the desirable traits are heritable. The present study provides information of the genetic parameters such as variance, coefficients of variation, heritability, genetic advance and nature of association between yield and its components to help to evolve suitable cultivars. Path coefficient analysis was performed to qualify direct and indirect contributors of yield components to grain.

MATERIALS AND METHODS

Nineteen genotypes of sorghum were evaluated in a randomized block design with three replications during *kharif* 2008 at College of Agriculture, RVSKVV, Gwalior. Each genotype was sown in six rows by adopting a spacing of 45 x 15 cm. Observation on days to 50% flowering, plant height (cm), leaf length (cm),leaf width (cm), width of panicle at maturity (cm), length of panicle at maturity (cm), 1000 grain weight (g), grain yield/ plant (g) and fodder yield/ plant were recorded in 10 randomly selected plants in each replication for each genotype. Phenotypic and genotypic coefficient of variation (PCV and GCV) was calculated based on the formula given by Burton, 1952, heritability and Genetic advance as per cent of mean by Johnson, *et al.,* 1955, path analysis by Dewey and Lu, 1959. Phenotypic and genotypic correlation coefficients by Johnson, *et. al.,* 1955.

RESULTS AND DISCUSSION

All characters studied showed wide range of genetic variability for all character. Genotypic coefficient of variation (GCV) was maximum for grain yield/plant (27.49) and its

Table 1. Estimates of Phenotypic and Genotypic Correlation Coefficients

Characters		Plant height at maturity	Number of leaves per plant	Leaf length	Leaf width	Length of panicle at maturity	Width of panicle at maturity	Fodder yield/plant	1000 grain weight	Grain yield/plant (g)
Days to 50 % flowering	P	0.321	0.109	-0.256	-0.241	0.375	0.254	0.198	0.134	-0.330
	G	0.352	0.119	-0.293	-0.185	0.405	0.221	0.273	0.212	-0.414
Plant height at maturity	P		0.103	0.285	-0.136	0.142	0.111	0.297	0.170	0.369
	G		0.206	0.316	-0.152	0.205	0.187	0.304	0.228	0.414
Number of leaves per plant	P			0.135	-0.050	0.265	0.253	0.384	0.060	0.291
	G			-0.190	0.138	0.303	0.293	0.484*	0.104	0.210
Leaf length	P				0.252	0.131	-0.276	0.081	-0.398	0.226
	G				0.285	-0.189	-0.343	-0.154	-0.352	0.240
Leaf width	P					0.115	-0.079	-0.132	-0.300	-0.171
	G					0.251	-0.132	0.242	-0.399	-0.184
Length of panicle at maturity	P						0.331	0.354	0.268	0.293
	G						0.378	0.384	0.397	0.372
Width of panicle at maturity	P							0.213	0.291	0.270
	G							0.244	0.326	0.242
Fodder yield/plant	P								0.124	0.344
	G								0.185	0.519**
1000 grain weight (g)	P									0.488*
	G									0.635**

*Significant at p=0.05 **Significant at p=0.01

Table 2. Phenotypic and Genotypic Path Coefficients showing direct and indirect effects taking grain yield per plant as dependent variable

Characters		Days to 50 % flowering	Plant height at maturity	Number of leaves per plant	Leaf length	Leaf width	Length of panicle at maturity	Width of panicle at maturity	Fodder yield per Plant	1000 grain weight	Phenotypic and Genotypic Correlation of Grain yield
Days to 50 % flowering	P	**0.128**	0.064	-0.196	-0.048	-0.033	-0.131	0.146	0.068	-0.139	-0.330
	G	**0.133**	0.087	-0.232	-0.151	-0.119	0.172	0.156	0.210	0.160	-0.414
Plant height at maturity	P	-0.80	**0.153**	0.138	0.059	0.082	-0.207	-0.182	0.268	0.214	0.369
	G	-0.058	**0.164**	0.193	-0.187	0.132	-0.215	-0.201	0.355	0.171	0.414
Number of leaves per plant	P	-0.111	0.316	**-0.071**	-0.014	-0.043	0.160	-0.232	0.198	-0.130	0.291
	G	-0.162	0.310	**-0.027**	-0.043	-0.084	0.158	-0.283	0.205	-0.126	0.410
Leaf length	P	0.034	-0.044	0.162	**-0.032**	0.215	-0.112	-0.049	0.228	-0.146	0.266
	G	0.031	-0.100	0.129	**-0.051**	0.269	-0.163	0.077	0.332	-0.186	0.250
Leaf width	P	0.231	-0.005	0.019	-0.008	**-0.060**	0.020	-0.058	-0.011	0.035	-0.171
	G	0.276	-0.161	0.100	0.131	**0.011**	-0.272	0.224	-0.305	0.156	-0.184
Length of panicle at maturity	P	0.074	0.106	-0.124	-0.120	-0.147	**0.178**	0.136	0.336	0.125	0.293
	G	0.107	0.126	-0.129	-0.168	-0.155	**0.220**	0.164	0.359	0.124	0.372
Width of panicle at maturity	P	0.138	0.217	-0.108	-0.115	-0.105	0.098	**0.133**	0.405	0.222	0.370
	G	0.163	0.218	-0.136	-0.183	-0.164	0.105	**0.140**	0.437	0.233	0.270**
Fodder yield/ plant	P	-0.185	0.130	0.226	0.103	-0.052	0.219	0.150	**0.545**	0.203	0.242
	G	-0.168	0.154	0.238	0.116	-0.087	0.236	0.127	**0.654**	0.233	0.519**
1000 grain weight (g)	P	0.143	0.218	-0.196	-0.113	-0.118	0.239	0.240	0.218	**0.316**	0.488**
	G	0.180	0.266	-0.194	-0.147	-0.105	0.279	0.211	0.227	**0.313**	0.635**

Phenotypic Residual Effect = 0.188 Genotypic Residual Effect = 0.069

difference with phenotypic coefficient of variation (PCV) was found less (27.66). Differences between GCV and PCV for other traits were also found to be less, indicating that these traits were less affected by environment. High heritability coupled with high genetic advance was observed for plant height at maturity and fodder yield/plant indicating that these characters are controlled by additive gene action and phenotypic selection for these characters will be effective. High heritability and high genetic advance for plant height have been reported by Wankhede, *et al.,* 1985, Umakanth, *et. al.,* 2003 and Mahajan, *et.al.,* 2011.

The phenotype of plant is the result of interaction of a large number of factors. Therefore, final yield is the sum total of component characters. The estimates of phenotypic and genotypic correlation coefficients worked out among different characters (Table 1) revealed that genotypic correlations coefficients were higher than phenotypic correlations. High genotypic correlation suggested that there was inherent relationship between characters under study. Fodder yield / plant and 1000 grain weight were found to be significant and positively correlated with Grain yield/ plant. Similar results were also reported by Umakanth, *et. al.,* 2004. Dry fodder is also important aspect of sorghum cultivation. It was found that No. of leaves/plant showed significant positive correlation with dry fodder yield /plant.

Genotypic and phenotypic correlations were further partitioned in to direct and indirect effects to know the relative magnitude of yield contributing components (Table 2). The results of path analysis revealed that days to 50% flowering, plant height, length of panicle, width of panicle, fodder yield/ plant and 1000 grain weight had positive direct effect on grain yield/plant. Fodder yield/plant had highest direct positive effect on grain yield. Its correlation with yield was also positive significant. It is revealed from the present study that the traits like plant height, 1000 grain weight and fodder yield/plant had greater importance. Hence, due consideration should be given to these characters while planning a breeding strategy.

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Prevalence of Arthropod Parasites in Bovines (Cattle and Buffalo) in Eastern Zone of Vidarbha Region

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ABSTRACT

Prevalence of arthropod parasites in bovines was investigated from villages of eastern zone of Vidarbha region (Districts-Bhandara, Gondia, Chandrapur, and Gadchiroli). The overall prevalence of infection in bovines of Bhandara, Gondia, Gadchiroli and Chandrapur districts was 79.39 , 71.40 , 79.71 and 78.23 per cent respectively. The effect of season on the prevalence of infection was studied in Mansoon, post monsoon, winter and summer season at eastern zone of Vidarbha region. The season-wise overall prevalence of arthropods parasitism in monsoon, post monsoon, winter and summer season was 77.24, 87.84, 78.73 and 70.78 per cent respectively. On statistical analysis the seasonal variation in the prevalence of infection in eastern zone was found highly significant (P < 0.01) in all four districts.

Key words: *Cattle and buffalo, season, breeds*

Bovines contribute an important role in strengthening the standard of rural living through a way as large milk producer, hides and hoof producer, manure producer etc. Arthropods are considered important ectoparasites of livestock as they are known to cause direct injuries. None of the domesticated animals are free from attack of ectoparasites. Apart from the direct infestation, the arthropod also transmit blood protozoan diseases *viz.,* trypanosomosis, babesiosis, theileriosis, anaplasmosis, ehrlichiosis etc.

MATERIALS AND METHODS

The present study was undertaken to ascertain the prevalence in bovines of eastern zone of Vidarbha region (Districts-Bhandara, Gondia, Chandrapur and Gadachiroli). A total 1200 bovines incluiding cattle and buffaloes of different breeds were examined. Ectoprasites such as ticks, lice and flies were collected from different parts of body into bottle containing 70% alchohol and 10% formaline solution and transported to laboratory for identification . The preserved ticks and flies were routinely examined under low power microscope for gross identification. The specimen glass bottle containing scrapping was gently heated to a temperature just sufficient to be tolerated on back of hand (about 38°C) and examined under dissecting microscope for the presence of live mites ,which showed motility in positive case. The scrapping found negative for infestation by above examination were transferred to the test tube to which 10 ml of 10% potassium hydroxide (KOH) was added and heated for 3-5 minutes in a beaker containing water, later on the tubes were centrifuged for 2 minutes at 1000 rpm .The supernatant fluid was discarded and last drop of sediment was taken on slide and examined microscopically for the presence of mites and their eggs.

RESULTS AND DISCUSSION

Out of 2241 animals examined 1732 were found positive for arthropod parasitism (77.29%). The range being minimum of 59.77% in May to a maximum of 89.62% in November at Eastern zone of Vidarbha region. The variation in types of arthropod infestation in bovines of eastern zone of Vidarbha region revealed 28.57% ticks, 22.92% flies, 7.10% lice, 1.67% mites and 39.72% mixed infection. Narwade and Maske, 2000 reported the mixed arthropods infestation 82.36% in dairy animals of Vidarbha region (M.S.) The effect of season on types of arthropods infestation showed maximum prevalence of ticks. The overall prevalence of infection in bovines of Bhandara, Gondia, Gadchiroli and Chandrapur districts was 79.39, 71.40, 79.71 and 78.23 per cent etc. The season-wise overall prevalence of arthropods parasitism in monsoon, post monsoon, winter and summer season was 77.24, 87.84, 78.73 and 70.78 per cent respectively. On statistical analysis the seasonal variation in the prevalence of infection in eastern zone was found highly significant (P < 0.01) in all four districts. In Bhandara the prevalence was 77.42, 88.46, 78.42 and 77.27 percent respectively, in Gondia the prevalence was 79.51, 88.89, 70.59 and 57.32 per cent respectively, in Gadchiroli the prevalence was 81.18, 84.74, 79.76 and 75.89 per cent

Fig. 1. Photograph of ticks infestation in calf

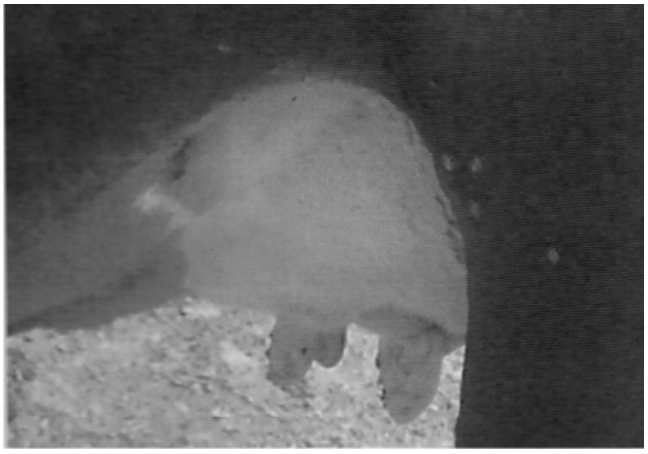

Fig. 2. Photograph of buffalo having *Hippobosce* flies infestation

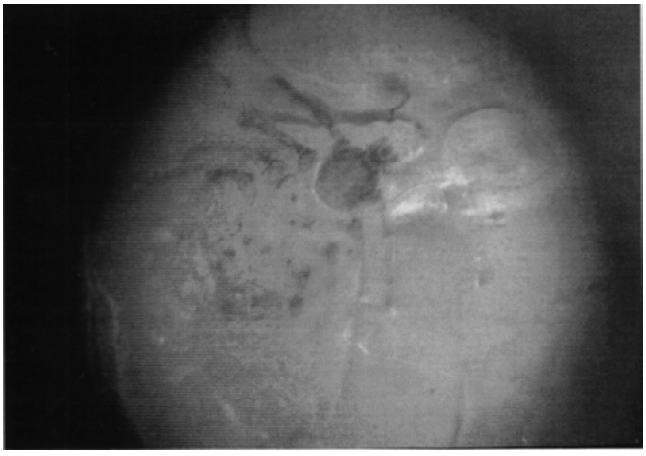

Fig. 3. Photograph showing *Rhipicephalus* sp. Tick

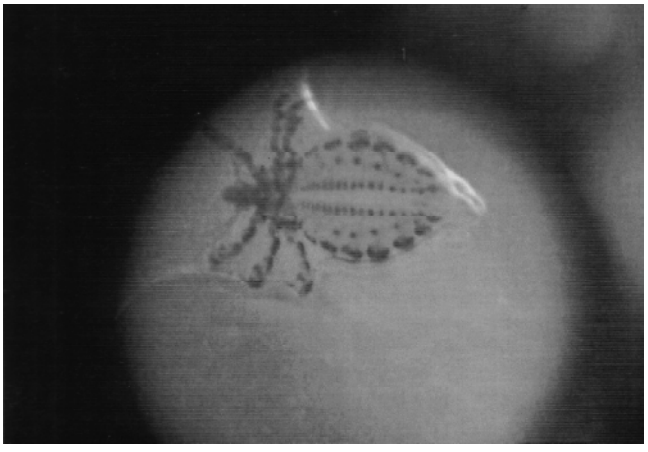

Fig. 4. Photograph showing *Haematopinus* sp. Lice

Fig. 5. Photograph showing *Stomoxys* sp. fly

respectively and in Chandrapur the prevalence was 73.68, 88.41, 82.52 and 73.48 per cent respectively. These observations corroborated with the findings observed by Maske, *et al.,* 1990, who stated that prevalence of mange on cattle in Nagpur was more during rainy and post monsoon, while flies and lies during post monsoon season. Singh and Chhabra, 1991 reported maximum incidence of lice and mites in winter and of ticks and flies in summer in Haryana. Sonkusale, *et al.,* 2004 reported prevalence of arthropod infestation highest in post monsson and winter season while least in summer season.

The prevalence of arthropod infestation in bovines of eastern zone of Vidarbha region was 77.28 per cent. Infestation was more in post monsoon season (84.86%) followed by winter, monsoon and summer. Prevalence of ticks was more than flies, lice and mites.

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Study of Physico-chemical Characteristic of Algal Flora (Chlorophyceae) of Sher Shah Suri Pond, Kanpur

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ABSTRACT

The physico-chemical characteristics of Sher Shah Suri pond were found variable according to the season. The study of occurrence and periodicity of algal sample revealed that distribution of green algae (chlorophyceae) were 15 out of 40 total algae recorded belongs to all the group of fresh water i.e. cyanophyceae, chlorophyceae and bacillariophyceae.

Key words: *Physico-chemical quality, Shershah suri pond, Algae, chlorophyceae.*

Sher Shah Suri pond in Kanpur is situated at 26.34 N latitude and 80.35E longitude at an elevation of 110 meters from level on the bank of river Ganga. It is also situated at adjacent most important national highways NH-2 on the main Delhi-Howrah Grand Trunk Road. The physico-chemical and biological characteristics of the pond reveal degrading condition of the ambient water, which can be felt on account of its obnoxious smell and greenish appearance. Asghar, *et al.,* 2010; Kumar, *et al.,* 2010; Munawar, 1970; Panday, 1973; Prasad and Saxena, 1980 were associated earlier with algal communities with varying degree of pollution. In the present study an attempt was made to correlate the distribution and periodicity of algae with chemical picture of pond water.

MATERIALS AND METHODS

Standard methods for the examination of effluent (APHA, 1976) were followed in the analytical techniques. A regular monthly sampling of effluent with simultaneous collection of algae was conducted. Spots were selected for collecting samples. Sampling was carried out from four corners of the pond. The samples were collected at the interval of one month. All the samples were brought to the laboratory and preserved at room temperature till the analysis was completed. The details of sampling procedure were same as described in Indian Standard methods of sampling and test of water.

The collected samples were analysed for different variables by the standard methods. From the preserved sample, algal material were mounted on slides and examined in detail and identified up to genus level with standard literature.

Table 1. Physico-chemical characteristics of Sher Shah Suri pond-water, Kanpur

Parameters	2011											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Temp.(°C)	17	22	26	30	33	32	33	32	30	25	24	16
pH	7.8	8.2	8.1	7.8	8.2	7.3	7.9	8.18	8.26	8.12	7.8	7.2
DO(mg/l)	7.3	7.1	7.2	7.5	6.4	5.9	6.1	8.5	7.1	7.9	4.1	4.8
BOD(mg/l)	4.9	5.7	6.5	5.9	12.5	8.7	13.5	5.8	4.7	4.9	7.5	5.2

Table 2. Distribution pattern of algae (Chlorophyceae) in Sher Shah Suri pond-water, Kanpur

S.N.	Algae genera	2011											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1.	*Actinastrum*	+	+	+	+	+	-	-	-	+	+	+	+
2.	*Cosmarium*	+	+	+	+	-	-	-	-	+	+	+	+
3.	*Chlorococcum*	+	+	+	+	+	-	-	-	+	+	+	+
4.	*Chlorella*	+	+	-	+	-	-	-	+	+	+	+	+
5.	*Cladophora*	+	+	-	-	-	-	-	+	+	+	+	+
6.	*Closteriopsis*	+	+	+	-	-	-	-	+	+	+	+	+
7.	*Closterium*	+	+	+	-	-	-	-	-	+	+	+	+
8.	*Hydrodictyon*	+	+	+	+	+	-	-	-	-	+	+	+
9.	*Microspora*	+	+	-	-	-	-	-	-	-	+	+	+
10.	*Oedogonium*	+	+	+	-	-	-	-	-	-	+	+	+
11.	*Oocystis*	+	+	+	-	-	-	-	-	-	+	+	+
12.	*Pediastrum*	+	+	-	-	-	-	-	-	-	-	+	+
13.	*Spirogyra*	+	+	+	-	-	-	+	-	-	+	+	+
14.	*Stigeoclonium*	+	+	+	-	-	-	-	+	+	+	+	+
15.	*Zygnema*	+	+	-	+	+	-	-	-	+	+	+	+

(+) = Present as abundant algae, (–) = Not present as abundant algae.

RESULTS AND DISCUSSION

Annual average values of important chemical parameters are given in Table 1. The occurrence and periodicity of algal samples studied are given in Table 2. In the present study only algae belongs to family chlorophyceae were undertaken. Waters favouring green algae are chemically distinct. In the present study green algae are consisting of fifteen genera with dominance of *Hydrodictyon* over *Cladophora*. *Spirogyra* was found to be abundant throughout the year. The abundance is attributed to favourable contents like less dissolved oxygen, oxidizable organic matter and absence of high water currents.

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In Vitro Evaluation of Anti Microbial Activity of *Calophyllum inophyllum* Linn

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ABSTRACT

An attempt was made to assess the anti microbial activity of *Calophyllum inophyllum* Linn in vitro condition using acetone extract of leaf and stem by Agar well diffusion method. *Staphylococcus aureus, E. Coli and Micrococcus* sp., were used as test bacteria for assessing anti bacterial activity. The leaf and stem extract were found to possess good anti bacterial properties.

Key words: *In vitro, antimicrobial, Calophyllum inophyllum*

Calophyllum inophyllum Linn oil yielding tree naturally growing on seashores and salty lands of western *ghats* of India. It is also found in Asia, Africa and Pacific region, now cultivated in garden as a ornamental tree.

The plant possess several alkaloids and phytochemicals such as Friedelin, B sitosterol, B amyrin, canophyllol, calophylloids, inophylloie, and calophyllic acid from ripe seeds. Cinnamic acid, inophyllic and calophsaic acid from unripe seeds.Myricetin glucosid, leucocynanidin from flowers. The leaves contain saponin and hydrocyanic acid.

Leaves are used in the treatment of chicken pox, scabies, sunburn, skin inflammation. Bark is used as astringent, diuretic, ulcers etc., Seeds are used in skin rash, rheumatism and as vermifuge. It is a highly esteemed external application in rheumatism and also in gonorrhea and scabies.

The plant having so much importance in the field of Ayurveda, homeopathy and as traditional medicine. Therefore, an attempt was made to study its anti microbial properties of leaves and stem in acetone extract in the laboratory against some bacteria.

MATERIALS AND METHODS

Freshly harvested leaves and stem of *Calophyllum inophyllum* Linn were collected from western *ghat* (Dev gad) Maharashtra State for experimental study. The collected samples were brought to the laboratory, thoroughly washed in tap water and later by distilled water, cut in to small pieces. Initially these small pieces were shade dried for 48 hours, later dried in electric oven with a temperature of 55-58º C for consecutive 4 days. The dried samples were then powdered with home grinder. This 20 g of fine powder of leaves and stem were subjected for extraction. Acetone used as solvent and extraction was carried out by Soxhlet's apparatus. Each of these extracts were further evapourated by Rotary vaccum evaporator till a gummy semi solid material was obtained. This semi solid material is used for studying anti bacterial properties.

While studying anti bacterial activity the test bacteria such as *Staphylococcus aureus, E.coli and Micrococcus* sp., were obtained from Department of Microbiology, Shivaji University, Kolhapur and were maintained in nutrient agar media. The bacterial suspension was prepared using saline water and mixed with 100 ml of sterilized nutrient agar media with constant shaking. This 20 ml seeded media was transferred to sterile petriplates. After solidification, well or cup was scooped with the help of cork borer 5 mm diameter. The test solutions were poured in the wells with the help of sterilized pipettes. Anti bacterial activity was carried out by agar well diffusion method (Alice and Sivaprakasm, 1966). The cultures were kept in incubator 28ºC for 48 hours and inhibition zone was recorded in mm.

RESULTS AND DISCUSSION

The results were depicted in Table 1 Among the three bacterial species tested, a maximum zone of inhibition was recorded in leaf extract of *Calophyllum inophyllum* Linn 20.1 mm against *Staphylococcus aureus*, followed by *E.coli*. Acetone solvent exhibited the highest anti bacterial potency against bacteria (Table.1). A parallel result was recorded by Nagaraja, *et al.,* 2010 in *Orobanche aegyptiaca*. While comparatively a significant zone of inhibition 20.1 mm recorded in acetone extract of stem against *E.coli* sp., followed by *Staphylococcus aureus* . A similar finding was documented by Nagaraja, *et al.,* 2011 in *Zanthoxylum rhetsa* (Roxb) DC. A negligible zone of inhibition was recorded against *Micrococcus* sp. such finding were observed by Nagaraja, *et al.,* 2008 and Shimpi, *et al.,* 2005. The leaf extract in acetone proves to be possess good anti bacterial property. Therefore, the present study may helpful in preparing formulation of the plant product and may be used as eco-friendly management of plant diseases.

Table 1. Acetone extract of *Calophyllum inophyllum* Linn. against some bacteria

Test bacteria	Zone of inhibition in mm (diameter)		
	Control	Leaf	Stem
Staphyllococcus aureus	4.1	20.1	19.2
Micrococcus sp.	5.1	15.1	14.0
E. coli	5.1	18. 0	20.1

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Comparative Performance of Different Nozzles on Deposition of Entomopathogenic Nematodes on Chickpea Leaves

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ABSTRACT

An experiment was conducted to evaluate the performance of different types of nozzles fitted with hand compression knapssac sprayer on deposition of *Steinernema sayeedae, S. seemae, S. qazii*. Five common nozzles *viz.*, flood jet nozzle, flat fan nozzle, broad cone nozzle, hollow cone nozzle (plastic type) and hollow cone nozzle (brass type) were taken in this study. EPNs @ 50,000 IJs/lit. of water was sprayed on 8 chickpea leaves kept in a plastic trays. The number of live EPN deposited on each leaf was counted. Maximum EPN was deposited in case of flood jet nozzle (607 IJs/leaf) showing its superiority over others as this nozzle resulted in least shredding and clogging of EPNs. This was followed by flat fan nozzle, while broad cone nozzle and hollow con nozzle were found very detrimental to EPNs.

Key words: *Steinernema sayeedae, S. qazii, S. seemae, nozzle, shredding, chickpea, Knapssac spray,*

Microbial bio-pesticides have become a key component of integrated pest management (IPM) practices. The concern for the environmental impact of alien invasive species will further increase demand for biological control. Meeting the demands of stakeholders will require further development and harmonization of international protocols, especially to non-target organisms and adverse ecological effects. In pulses ecosystem various microbial bio-pesticides like *Nuclear Polyhedral Virus* of *Helicoverpa armigera* (*HaNPV)*, entomogenous fungi, entomopathogenic nematodes (EPNs) etc., have shown good promise as these are effective to a variety of insect pests, easy to multiply, economical, environment friendly and there is good scope for their commercial production. They are recognized potential bioagents against a number of agriculturally important insect pests (Kaya and Gaugler, 1993, Ali, *et al.,* 2005a, 2005b)

Being different sizes of EPN (Table 1) it is prerequisite to know that which available nozzle can give best deposition of EPN on chickpea leaves used in hand compression sprayers for aerial spray for the management of *Helicoverpa armigera*. These nozzles are commonly used in the country for aerial spray of insecticides, fungicides, weedicides and nematicides. Some times the nozzle is blocked during spray, then whole spray operation has to be stopped until nozzle has to be cleared. In view of this, the present studies were carried out with respect to EPN sprays to find out which nozzle performed better in terms of coverage on leaf surface during aerial spray of EPNs are emerging potential control agent for the pest, specially lepidopterons and other group of insects pest on Chickpea, Pigeonpea, Cotton and has added a new diamention to IPM strategies. When these enter to the insect body through natural openings they release bacteria which produce toxin and kill the insect, thereby providing a good medium for multiplication of EPN. Entomopathogenic nematodes of the genus *Steinernema* spp. and *Heterorhabditis* spp. are symbiotically associated with bacteria of the genus *Xenorhabdus* and *Photorhabdus* respectively. EPNs are promising biocontrol agents alternative to chemical insecticides, 68.6% increase in yield of chickpea was obtained by spraying *Steinernema masoodi* against *Helicoverpa armigera* (Ahmed, *et.al.,* 2009). Application of EPN liquid formulation containing $3x10^5$ IJS/ha through hand compression knapssac sprayer using flood jet nozzle on pigeonpea short duration variety, UPAS 120, infested with *H. armigera,* the increase in yield was 18.9% over untreated control (Ali, *et.al.,* 2010). To test the performance of different nozzles used in hand compression knapsae sprayer for liquid formulation of different *Steinernema* species used in EPN biopesticide for their coverage; the present study focus on proper and adequate distribution of nematodes on leaf surface to achieve the target.

MATERIALS AND METHODS

An experiment was conducted to evaluate the performance of different types of nozzles on deposition and coverage of leaf area by EPNs as well as shredding inside different nozzles used in hand compression knapssac sprayers. Liquid formulation of *S. sayeedae (*Ali and Shaheen, 2011*), S. qazii (*Ali, *et al.*,2009*), S. seemae (*Ali, *et al.*, 2005 a*)* on Chickpea leaves were studied when applied through hand compression sprayer. Five common nozzles *viz*., flood jet nozzle, flat fan nozzle, broad cone nozzle, hollow cone nozzle (plastic type) and hollow cone nozzle (brass type) were studied. Nuclear culture of *S. sayeedee*, *S. seemae*, *S. qazii* obtained from mother culture in the laboratory. Final instar larvae of waxmoth, *Galleria mellonella* (L.) reared on artificial diet (David and Kurup, 1988) and field collected larvae of pod borer,

Helicoverpa armigera were used as laboratory hosts for multiplication of ENPs. The basic *in vivo* production method outlined by Woodring and Kaya, 1988 was followed for multiplication, storage and quantification of the population of the nematodes. Spray of EPNs @ 50,000 IJs/lit. of water was carried out on 8 chickpea leaves kept on a plastic tray. A thorough coverage of leaves by maintaining uniform pressure was done for the deposition and coverage of live EPN on leaves and immediately these were dipped into beaker containing distilled water. The number of live EPNs deposited on each leaf was counted under steareozoom microscope. Photographs of nozzles were taken (Fig. 1) to observe the EPNs sticking from inside and clogging of nozzle by EPNs. There were five treatments, replicated eight times and arranged in CRD, Counting was done under steareozoom microscope.

RESULTS AND DISCUSSION

Maximum number of *S. sayeedae, S. seemae* and *S. qazii* were deposited on chickpea leaves by flood jet nozzle (Table 2) showing its superiority over others. This nozzle generally used for weedicide spray. About 50% reduction in deposition of EPNs were observed in flat fan nozzle and broad cone nozzle as compared to flood jet nozzle. These nozzles are generally used for the spray of insecticide and fungicide sprays. Hollow cone nozzle either plastic type or brass type were found very detrimanlet to EPNs where only 25% of EPN were found deposited on chickpea leaves as compared to flood jet nozzle.

Microscopic observations of these inverted nozzles indicated that maximum shredding and clogging of nozzles were seen in Hollow Cone Nozzles where a number of EPNs

Table 1. Length and width of EPNs used in Liquid formulation for spraying on Chickpea leaves (measurement in µm)

Steinernema	3rd stage juvenile			Male			Female			Giant female	
	Length (µm)	Width (µm)	'a' value	Length (µm)	Width (µm)	'a' value	Length (µm)	Width (µm)	'a' value	Length (µm)	Width (µm)
S. sayeedae	372-425	220-270	18-19	956-1131	60-62	13	1188-1455	60-85	19-23	3421-4332	198
S. seemae	380-400	22-24	17.4	702-808	54-71	129-18	1241-1538	84-97	13.7-17.6	4305-6014	217
S. qazii	420-425	21-23	21.7	829-936	48-49	16-18.9	936-1067	50-92	15-21	-	-

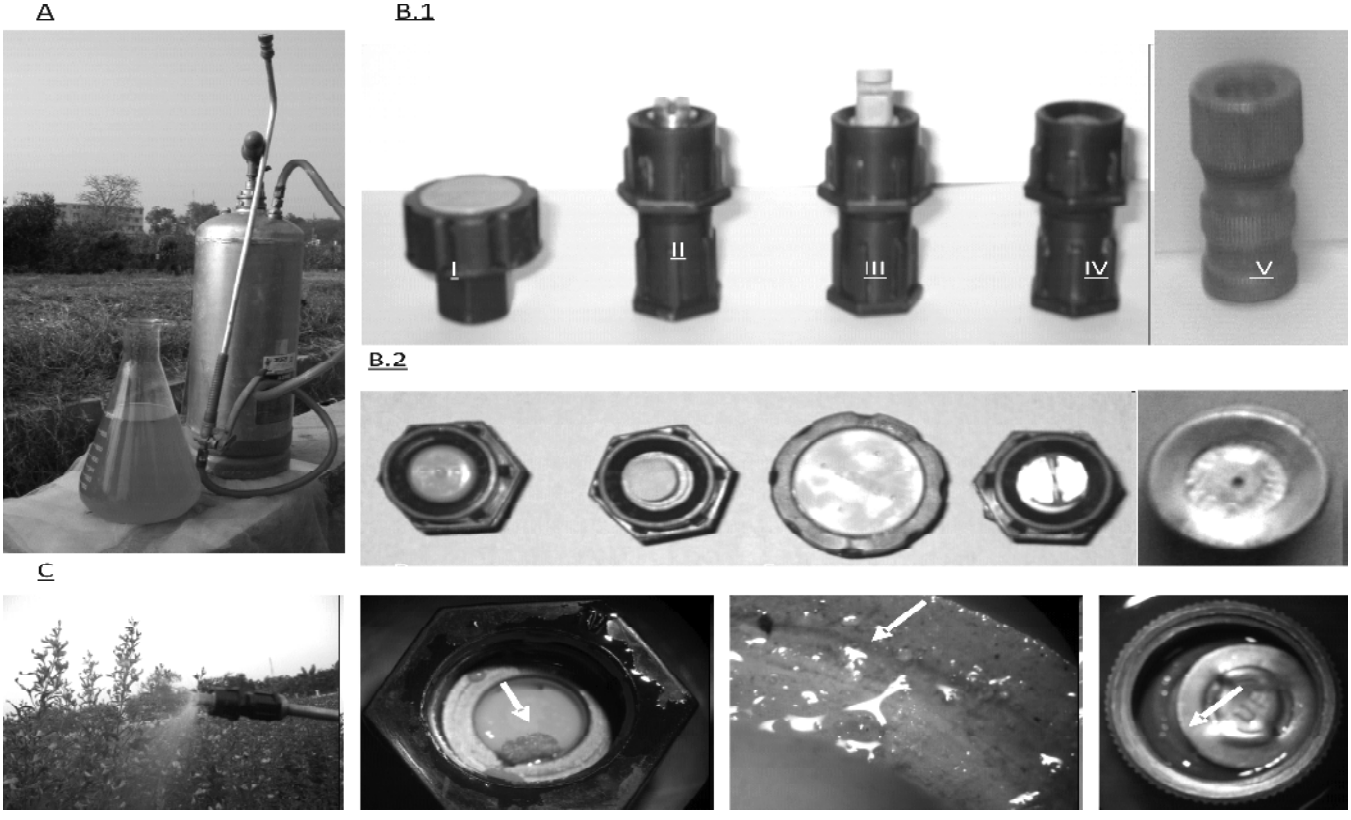

Fig. 1. Nozzles and EPN based biopesticide spray. A. Hand compression knap sac soprayer, Liquid formulation of EPN biopesticide in flask and chickpea crop B.1 Different nozzles – Front view B.2 Different nozzles – Top view i. Broad cone nozzle ii. Flat fan nozzle iii. Flood jet nozzle iv. Hollow cone nozzle (plastic type) v. Hollow cone nozzle (brass type) C. Spraying liquid formulation of EPNs biopesticide with flood jet nozzle D, E, F. EPNs sticking/shredding inside the nozzles after spray.

Table 2. Mean no. of EPNs deposited on Chickpea leaves during spray through different nozzles using different *Steinernema* species.

Nematodes (*Steinernema* spp.) (Mean nos.)			
Nozzle	***S. sayeedae***	***S. qazii***	***S. seemae***
Flood jet nozzle	570.233	570.06	607.96
	(61.15)	(54.04)	(62.64)
Flat fan	233.83	236.40	249.13
	(35.18)	(32.56)	(39.45)
Broad cone	211.76	212.73	232.06
	(51.30)	(45.13)	(30.13)
Hollow cone plastic	129.83	139.60	144.43
	(26.57)	(27.18)	(22.87)
Hollow cone brass	139.63	144.73	137.96
	(18.99)	(19.91)	(24.36)

(Figures in parenthesis are S.D. values)

clogged in bunches and closed the pores of nozzle (Fig.1) hence, less deposition of EPNS on leaves was observed while flood jet nozzle showed no such cluster of EPN's and clogging the pores of nozzle. Hence flood jet nozzle was found best for EPNs liquid formulation for spraying, while the flat fan and broad cone nozzle, hollow cone nozzle are not suitable for spray of EPNs liquid formulation as cloging results shredding and mortality of EPNS inside these nozzles. These nozzles are found detrimental to EPNs except Flood Jet Nozzle (Table 2).

Standard spraying system that are designed for chemical application donot perform very efficiently when applying particulate material such as nematode IJs (Lello, *et al*., 1996). Our studies also corroborates with the studies of Lello, *et al*., 1996, however while, using of flood jet nozzle only promising results with EPNs have been achieved. Hydrautic nozzle (flat fan, full cone) produces a wide range of droplets many of which are too small to carry on IJs and therefore have a higher water to nematode ratio. Higher output (flow rate) (IJs/cm^2 of leaf) and in laboratory studies greater insect control (Lello, *et al*., 1996).

It is concluded that maximum EPN was deposited in case of flood jet nozzle showing its superiority over others, as this type of nozzle resulted in least shredding of EPN, this was followed by flat fan nozzle and broad cone nozzle. Hollow cone nozzle either plastic type or brass type were found very detrimental to EPNs when only (129 IJs/leaf) were deposited and rest got shredded or destroyed (Table 2).

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Biocomposting of *Jatropha* De-oiled Cake

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ABSTRACT

***Jatropha curcus* is receiving a lot of attention as biofuel energy crop. In addition to seed oil, it also produces wood, fruit shells, seed husks and press cake. The de-oiled seed cake of *Jatropha* is a major by-product of biodiesel extraction process, which is rich in organic matter. In the present study, two combinations with *Jatropha* de-oiled cake were taken for the preparation of bio compost and they were assessed for their macro and micro nutrient content, which can be supplemented to the plants for their nutritional requirement. It was found that both composts were having the desired level of nutrient content. It was concluded that these biocompost made from waste de-ioled cake of *Jatropha* can be used as a valuable source of stable organic matter and major nutrients for plants.**

Key words: *Jatropha curcus, de-oiled cake, biocompost, nutrients.*

Jatropha curcas has attained worldwide interest because of biofuel production. Besided biofuel production, *Jatropha* plant has several more desirable properties such as hardiness, wide environmental tolerance. It can grow on any type of soil, adapt well to waste land easy propagation, carry high oil content and require minimal care. It also has less gestation period, rapid growth and is not browsed by animals. Because of these properties, this plant provides ample opportunities for wealth generation among marginal sections of the society and rural employment (Agarwal, *et al.,* 2007).

Jatropha can also be used as manure, as feedstock for biogas production and as animal feed. It contains about 30% oil leaving behind de-oiled cake. The de-oiled seed cake of *Jatropha* is a major by-product of bio-diesel extraction process, which is rich in organic matter (Abreu, 2009). Conventionally, *Jatropha* oil cake is also being used for soil enrichment (Reyadh, 1997). According to one study, in which *Jatropha* de-oiled cake was assessed for its suitability as substrate for enzyme production by solid state fermentation, it was found that the seed cake support good bacterial growth and enzyme production (protease, 1818 U/g of substrate and lipase, 625 U/g of substrate). This result demonstrated viable approach for utilization of this huge biomass for production of industrial enzyme (Mahanta, *et. al.,* 2008). It was also estimated in this study that *Jatropha* plantation can produce 1000kg seedcake/ hectare during biodiesel production.

During the era of environment safety, various forms of biomass such as different vegetation, animal dung and plant products along with *Jatropha* de-oiled cake are providing safe and convenient sources for the production of biocompost (Ali, *et. al.,* 2010). Considering 25% oil yield, an oil extraction plant of capacity 10,000 ton per year will produce 7500 ton of de-oiled cake every year. This easily available alternative resource can be harnessed by anaerobic composting to produce biocompost as an efficient waste management technique.

In the present study, two combinations with *Jatropha* de-oiled cake were taken for the preparation of bio compost and they were assessed for their macro and micro nutrient content, which can be supplement crops for their nutritional requirement.

MATERIALS AND METHODS

The traditional method of anaerobic composting was practiced during the year 2010 and pits were prepared in the month of July at Aromatic & Medicinal Research and Development Centre of G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India. In one pit of 4 cubic feet one quintal *Jatropha* de-oiled cake was placed along with 10 kg *Aloe vera* leaves and covered with polythene sheet. The pit was finally covered with straw and soil to provide anerobic conditions. The second pit was filled with 10 kg *Aloe vera* leaves, 5 kg cow-dung and 100 kg *Jatropha* de-oiled cake.

Both the materials were allowed to compost for 4 months. Samples were collected when the bad odour ceased completely and then it was examined for the nutrient contents as nitrogen, phosphorous, potassium, zinc, copper, iron and Manganese as per the established methods (Jackson, 1973).

RESULTS AND DISCUSSION

Jatropha seed cake makes an excellent organic fertilizer with high nitrogen content. The macro and micro nutrient composition of test samples used in the study are given in Table 1.

Table 1. Macro and Micro-nutrient contents of *Jatropha* de-oiled cake along with different composition

S.N.	Type of Compost	N (%)	P (%)	K (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
1.	*Jatropha* de-oiled cake + *Aloevera*	2.5	0.2	41.2	0.774	0.124	49.44	2.031
2	*Jatropha* de-oiled cake + *Aloevera* + Cow-dung	3.2	0.16	51	0.392	0.110	32.70	0.930

Fig. 1. Sample 1 (*Jatropha* de-oiled cake + *Aloe vera*) biocompost.

Fig. 2. Sample 2 (*Jatropha* de-oiled cake + *Aloe vera* + Cow-dung) Biocompost.

Generally *Jatropha* oil cake alone contain N % of 1.80 while in the samples studied N% was found to be 2.5 and 3.2. Thus it was higher for the sample where *Jatropha* de-oiled cake along with aloe-vera and cow-dung was used for the preparing biocompost. The content of micro elements *viz.,* Zn: Cu: Fe: Mn (in ppm) in two types of compost were 0.774:0.124:49.44:2.031 and 0.392:0.110:32.72:0.930 respectively. When compared micronutrients, the Zn, Fe and Mn contents were higher in *Jatropha* deoiled cake + *Aloe vera* compost than *Jatropha* deoiled cake + *Aloe vera* + cow-dung compost, while the Cu content was almost same in both the compost types.

In the present study, nutrient content in finished compost was within the desired level whereas micro-nutrients such as copper, lead and nickel were much below the maximum allowable concentrations, which was in accordance with Das, *et. al.,* 2011.

The essential elements for the growth of any plant are nitrogen, phosphorous and potassium. All these elements play a significant role if used in the form of a manure (or) fertilizer. Since composting finally leads to organic manure it is worthwhile in estimating these elements in the composted material obtained after a period of 60 and 90 days (Janakiram and Sridevi, 2010). According to one study, effective use of some fibrous material like straw, leaves or wood chips which can absorb relatively large quantities of water and still maintain structural integrity and porosity could prevent the loss of potassium from the compost formed (Iyenger and Bhave, 2006). For this purpose, in the present study, *Aloe vera* leaves was taken along with *Jatropha* de-oiled cake, which can prevent usual potassium loss from the compost.

The recycling of organic materials to land is the best practicable environmental option in most circumstances, completing natural nutrient and carbon cycles. In the present study, the result of nutrient contents of two biocompost showed their potential to be a valuable source of stable organic matter and major plant nutrients. From this study, it can be concluded that different *Jatropha* biomass along with different combination can be utilized to produce bio-compost for the accomplishment of nutrients in soil.

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Survey and Occurrence of Leaf Spot of Brinjal Caused by *Alternaria alternata* (Fr.) Keissler in Jaipur District

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ABSTRACT

Brinjal (*Solanum melongena* L.) is a major vegetable crop of India. Leaf spot caused by *Alternaria alternata* (Fr.) Keissler was an important disease resulting into severe problems of this crops in the vicinity of Jobner in Jaipur districts of Rajasthan. Leaf spot disease intensity was observed upto 25.32%. Maximum disease intensity was observed in Bagru (25.32%) while minimum in Phulera (9.20%).

Key words: *Alternaria, Survey, Brinjal, Disease Intensity*

Brinjal (*Solanum melongena* L.) is a major vegetable crop of India. It is grown in all seasons and almost in all the parts of India except on higher altitudes. In India, brinjal is mainly grown in the states like West Bengal, Orrisa, Bihar, Gujarat, Maharastra, Andhra Pradesh, Karnataka etc. with an area of 530.3 thousand hectare and production of 8703.8 metric tonnes (Anonymous, 2004-2005). It contributes about 8.2% of the total production of vegetables in India. In Rajasthan, it is grown in all the districts where irrigation facilities are available with an area of 5522 hectares, production 23369 metric tonnes and productivity of 4.38 metric tonnes per hectare (Anonymous, 2004-05). It is mainly grown in Alwar (area 1104 ha and production 5763 mt), Kota (area 365 ha and production 8720 mt), Jaipur (area 562 ha and production 2003 mt), Tonk (area 94 ha and production 736 mt), Bharatpur (area 492 ha and production 548 mt), Baran (area 292 ha and production 819 mt) and Bundi (area 266 ha and production 648 mt) districts. In Jaipur, district area under brinjal crop is 10.17 % area and 8.57 % of the total vegetable production. The productivity of Jaipur district is less (Anonymous, 2004-05). Brinjal is extensively grown in surrounding area of Bagru, Chomu, Lalpura, Phulera and Renwal in Jaipur district.

The principle limiting factor in profitable cultivation of this crop in Rajasthan is attack of several diseases mainly caused by fungi which take heavy loss of the crop at all the stage of plant growth. One of the important factors which limit the productivity in Jaipur region is the use of contaminated seed which results in poor seed germination and in vitiation of various diseases including leaf spot caused by *Alternaria alternata* during crop development in the field.

A heavy infection of leaf spot of brinjal caused by *Alternaria alternata* (Fr.) Keissler was observed in the vicinity of the Jobner (Jaipur). The leaf spot of brinjal is characterized by affected young seedlings which become blighted, charred and mortality occures. Small, circular, brown, necrotic leaf spots with a chlorotic halo were formed, which gradually enlarged and coalesced causing withering and shedding of the leaves. Fruit lesions were small (± ½ cm), concentric, dark brown and sunken, becoming olivaceous due to spore formation, coalescing and some times covering the entire surface.

Leaf spot caused by *Alternaria alternata* is an important disease observed in a severe from since last few years at Jobner (Jaipur) and nearby area. Sudarshan Rao, 1975 stated that survey and surveillance form the basis for any successful plant protection strategy. Successful plant protection depends upon early detection of the disease incidence followed by timely adoption and application of preventive measures. Keeping in view the problem of survey of brinjal fields was conducted in the vicinity of Jobner during *Rabi* season to estimate the disease intensity of leaf spot caused by *A. alternata*.

MATERIALS AND METHODS

Brinjal fields located in different villages situated in the vicinity of Jobner i.e. Renwal, Chomu, Lalpura, Bagru and Phulera were surveyed and plants showing typical symptoms of leaf spot of brinjal were collected for isolation. The survey was conducted during November and December of *rabi* season for recording disease intensity of leaf spot of brinjal 80 to 85 dap (days after planting). Five villages were selected nearby Jobner where brinjal was grown and in each village, four fields were selected randomly. in each fields, 5 plots (1 x 1 sqm) were diagonally marked. Disease intensity was recorded

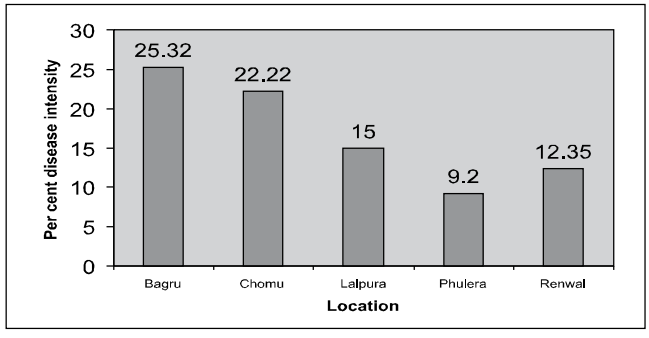

Fig. 1. Leaf spot intensity on brinjal caused by Alternaria alternate (Fr.) Keissler in rabi season

based on per cent leaf area covered by the disease symptoms (leaf spots) with the lays of disease rating key. One hundred twenty leaves (1/3 lower, 1/3 middle and 1/3 upper leaves) were selected at random at each location and per cent disease intensity was recorded using disease rating key given below:

Grade	Per cent leaf area infected
0	No infection (healthy)
1	1 to 25 per cent leaf area infected
2	25.1 to 50 per cent leaf area infected
3	50.1 to 75 per cent leaf area infected
4	More than 75 per cent leaf area infected

Per cent disease intensity (PDI) was calculated by using following formula (Mayee and Datar, 1986).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Number of leaves assessed} \times \text{Maximum disease rating}} \times 100$$

RESULTS AND DISCUSSION

The disease intensity was found varying from 9.20 to 25.32 with overall mean 16.81% from the different places surveyed in the vicinity Jobner (Jaipur). The maximum leaf spot disease intensity (25.32%) was recorded in Bagru followed by Chomu (22.22%) whereas minimum diseases intensity 9.20 and 12.35% observed in Phulera and Renwal, respectively (Fig. 1). Dinger and Singh, 1985 observed maximum disease intensity (33.87 and 34.31%) during second and first fortnight November of 1979 and 1980, respectively in Kanpur (U.P.). Singh and Shukla, 1986 observed maximum disease (39.12%) in 120 days old plant of brinjal infected with leaf spot caused by *Alternaria alternata* at Kanpur (U.P.).

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Combining Ability Analysis for Yield, Its Attributing Traits and Oil Content (%) in Indian mustard [*Brassica juncea* (L.) Czern and Coss]

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ABSTRACT

The experiment was carried out using diallel analysis excluding reciprocal comprised seven parents and their 21 straight F_1 hybrids, the material was grown in randomized block design with these replication during rabi at main Castor and Mustard Research Station Saradarkrushinagar (Gujarat). The ratio of gca to sca genetic variance for various characters indicated that, additive type of gene action was predominant for expressing traits like earliness, plant height, Length of silique, seed per silique and oil content The parent IC 385682 manifested good general combines for length of silique, 1000-seed weight and oil content.While SKM 0820 depicted good general combines for seed yield and oil content. Parent RGN 145 good general combines for seed per silique and seed yield. The cross combination IC 385682 x NDR 5-1, SKM 0820 x NDR 5-1, and NDCC28 x NDR 5-1 were found positive and significant sca effects for seed yield per plant. These combination for seed yield had involved A x P, G x P and A x G combiner. Additive and Non-additive type of gene effects, which is flexible in nature. Hence cross are expected through transgressive segregating with later generation.

Key words: *Combining ability, GCA, SCA, diallel analysis*

Mustard is the second most important group of oilseed crop in India after groundnut. This crop along with rapeseed account for 22.7 % of the total oilseed production and 19.2 % of the total cropped area in the country. At global level, it accounts for 23.5 % and 13.2% of the total hectares and production respectively. There has been remarkable increase in the production, which was hovering around 2.68 million tones with productivity level of 650 kg / ha until 1985-86 increased to 5.83 million tons in 2007-2008 while productivity increased to more than 1001 kg / ha. The important rapeseed mustard growing states in the country are Rajasthan, U.P, M.P, Haryana, Gujarat, West Bengal, Assam, and Bihar, Panjab and J& k. The mustard is predominantly self pollinated crop but some extent (5-18 %) cross pollination occur (Labana and Banga, 1984). The success of breeding produce is determined by the useful gene combination organized in the form of good combining lines and isolation of valuable germplasm. The diallel analysis furnish useful information on superior parents with their general and specific combining ability effects, Superior crosses may generate good recombinants in advance generation. Diallel analysis gives an overall genetic picture of the material in a single generation is an additional advantage.

MATERIALS AND METHODS

The experimental material included of seven parents and their 21 F_1 hybrids, the material was grown in Randomized Block Design with these replication during *rabi* at Main Castor and Mustard Research Station Saradarkrushinagar (Gujarat). The seven parents *viz.,* IC 385682, SKM 0820, NDCC 28, RLM 619, NDR 5-1, SKM 0450 and RGN 145 and their 21F_1 hybrids were planted individual in two row of 5 m length having an inter and intra row spacing of 45 x 15 cm, respectively. observations were recorded on 5 randomly taken plant from parents and F_1 in each replication for days to flowering, days to maturity, plant height (cm), length of main branch (cm), number of branches per plant, number of siliqua per plant, Length of silique (cm), seed per silique, 1000 seed weight (g), seed yield per plant (g) and oil content (%). Oil content was estimated using NMR method. Combining ability analysis was carried out by the method suggested by (Griffing, 1956) Mehod-2, and Model-1.

RESULTS AND DISCUSSION

The analysis of variance for combining ability (Table 1) revealed that, means sum of square for gca significant to highly significant for all the traits except days to flowering, days to maturity and number of branches per plant while the specific combining ability effects were significant for all the traits except days to maturity, length of main branches length of silique, seed per silique and oil per cent. The ratio of gca to sca genetic variance for various characters indicated that, additive type of gene action was predominant for expressing traits like earliness, plant height (Monpara and Dobariya, 2007) Length of silique (Singh, *et al.*, 2003), seed per silique and oil content (Srivastava, *et al.,* 2003).

General combining ability: General combining effects of parents (Table 2) revealed that none of the parents consistently good general combines for all the character under study. The parent IC 385682 good general combiners for length of silique, 1000 seed weight and oil content. SKM 0820 good general combines for seed yield and oil content. Parent RGN 145 good general combines for seed per silique and seed yield.

The good general combines parents had fixable component of variance like additive and epitasis component, these parent IC 385682, SKM 0820 and RGN 145 may be used for further hybridization program as a donor parent for

Table 1. Analysis of variance for combining ability for various characters in mustard

Source of variation	d.f.	Days to flowering	Days to maturity	Plant height	Length of main branches	Number of branches plant	Number siliqua per plant	Length of siliquae	Seed per siliquae	1000 seed weight	Seed yield per plant	Oil (%)
GCA	6	2.92	12.45	450**	45.21*	1.46	267.88	0.22**	1.51**	0.29**	270.96**	3.18**
SCA	21	5.33*	30.68	105.6*	30.09	2.77**	1055.71**	0.03	0.44	0.11**	313.23**	0.40
Error	54	2.70	30.17	59.73	18.16	0.83	369.62	0.02	0.32	0.03	42.86	0.67
σ^2 GCA		-0.27	-2.02	38.27	1.67	-0.14	-87.53	0.02	0.12	0.02	-4.69	0.31
σ^2 SCA		2.62	0.51	45.87	11.93	1.94	686.09	0.02	0.12	0.08	270.37	-0.27
σ^2 GCA/ σ^2 SCA		-0.10	-3.97	0.83	0.14	-0.08	-0.13	1.28	0.99	0.27	-0.02	-1.14

* and ** indicates significant at P = 0.05 and P = 0.01 levels, respectively.

Table 2. Estimation of general combining ability (GCA) effects of different characters

Sr. No.	Parents	Days to flowering	Days to maturity	plant height	Length of main branches	Number of branches plant	Number siliqua per plant	Length of siliquae	Seed per siliquae	1000 seed weight	Seed yield per plant	Oil (%)
1.	IC 385682	0.34	-1.41	-0.095	-1.34	-0.62*	-2.68	0.21**	0.17	0.37*	2.09	0.65*
2.	SKM 0820	-0.72	-1.56	-13.44**	-2.11	0.04	3.17	0.01	0.26	-0.05	8.28**	0.96**
3.	NDCC 28	-0.14	1.58	-0.42	0.68	0.64*	-0.61	-0.191**	-0.32	-0.11*	-0.66	-0.54*
4.	RLM 619	-0.59	-0.04	9.07**	-3.01*	0.04	0.77	-0.14**	-0.55**	-0.15*	-3.05	-0.15
5.	NDR 5-1	0.96	0.14	6.01*	3.03*	-0.36	-9.37	-0.13**	-0.39*	-0.05	-5.85*	-0.64*
6.	SKM0450	0.12	1.14	-0.15	0.57	0.09	8.52	-0.16**	0.31	-0.09	-6.04*	-0.11
7.	RGN 145	0.03	0.14	-0.97	2.20	0.18	0.19	0.07	0.51**	0.07	5.23*	-0.17
	S.E.(gi) ±	0.51	1.70	2.39	1.31	0.28	5.93	0.04	0.17	0.05	2.02	0.25
	S.E.(gi-gj) ±	0.78	2.59	3.64	2.01	0.43	9.06	0.06	0.27	0.08	3.09	0.39

* and ** indicates significant at P = 0.05 and P = 0.01 levels, respectively.

Table 3. Sca effects of three best crosses along with *per se* performance and gca combination for six characters in mustard.

Characters	hybrids	sca	gca	*per se* performance (Rank)
Grain yield	SKM 0820 x NDR 5-1	46.17**	G x P	117.97 **(1)**
per plant	IC 385682 x NDR 5-1	26.53**	A x P	92.17 **(2)**
	NDCC 28 x RGN 145	18.05**	Ax G	79.50 **(-)**
Days to flowering	IC 385682 x NDR 5-1	-4.09**	A x A	40.87 **(1)**
	NDCC 28 x RGN 145	-3.23*	A x A	40.93 **(2)**
	-	-	-	-
Plant height	SKM 0820 x RLM 619	-23. 81**	G x P	126 (1)
	-	-	-	-
	-	-	-	-
Number of branches per plant	IC 385682 x SKM 0820	2.64**	P x A	16.13 (1)
	-	-	-	-
	-	-	-	-
Number of siliqua per plant	IC 385682 x SKM 0820	40.64*	A x A	270.93 **(1)**
	SKM 0820 x NDR 5-1	38.86*	A x P	262.47 **(-)**
	SKM 0820 x RGN 145	34.50*	A x A	267.67 **(3)**
1000-seed weight	IC 385682 x NDCC 28	0.44*	G x P	4.54 **(1)**
	IC 385682 x SKM 0820	0.32*	G x A	4.47 **(2)**
	IC 385682 x SKM 0450	0.32*	G x A	4.43 **(-)**

G = Good; A = Average; P = Poor. * and ** indicates significant at P = 0.05 and P = 0.01 levels, respectively

improving the yield and its related characters in Indian mustard. The findings confirm earlier with Singh, *et al.,* 2006 and Singh and Dixit, 2006.

Specific combining ability: Analysis of specific combining ability is important parameters for judging the combination for exploiting it through heterotic breeding programme. Three best crosses selected on the basis of sca effects for each character presented in Table 3. On perusal of data revealed that none of the cross had high ranking sca effects for all the characters. The top ranking sca effects for most of the characters these accompanied by top ranking predominant role of non-additive gene effects in experiments seed yield and its components.

IC 385682 x NDR 5-1, NDCC 28 x RGN 145 for easily flowering , NDCC 28 x SKM 0450, SKM 0820 x RLM 619, NDCC 28 x NDR 5-1 for dwaefness, IC 385682 x SKM 820 for number of branches per plant, IC 385684 x SKM 0820, SKM 0820 x NDR 5-1, SKM 0820 x RGN 145 for number of silique

per plant. IC 385682 x SKM 0820, IC 385682 x NDCC 28, IC 385682 x SKM 0820 for test weigh, whereas for seed yield per plant positive and significant sca effects were found in F_1 hybrids IC 385682 x NDR 5-1, SKM 0820 x NDR 5-1, and NDCC 28 x NDR 5-1. None of the cross were found positive and significant effects for length of main branch, length of silique and oil content. The combination for seed yield had involved A x P, G x P and A x G combiner. Additive and Non-additive type of gene effects, which is flexible in nature. Hence crosses are expected.

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SHORT COMMUNICATION

Successful Treatment of Strangle in Horse

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Strangle is mainly a disease of equine caused by a bacteria *streptococci equi* . It is gram positive *Cocci baccilus* that produces a beta heamolysin, mortality rate is usually in younger horses such as foals. Sub- mandibular lymph nodes enlarged and phyrangitis may be so severe that the animal is unable to swallow and there is soft and moist cough and incubation period is one to three weeks . Fever is 103-105 degree Fahrenheit, anorexia etc. Previously strangle was reported by Sweeny, 1996, Timoney, 1993, Sisson and Grossman, 1953, Jorm, 1991.

The male pony showing the symptom of nasal discharge, fever and abscess of sub-mandibular lymph node in Vins Bio-Products Pvt. Ltd. Hydrabad, Company based on Antisera production from equines. Newton, *et al.,* 1997 reported naturally occurring persistent and symptomatic infection of the guttural pouches of horses with *Streptococcus equi*. From clinical symptoms the case was suspected for strangles . Blood samples collected from that horse and sent to the laboratory. Culture of *Streptococci equi* was done from the nasal and abscess discharges from that affected horse. On blood agar, a zone of clear haemolysis, surrounding colonies were observed.

The affected animal is treated with procaine penicilium G (22000IU / Kg IM in every 12h) for 5 days along with injection normal saline 1Lit. IV and inj. Ketoprofane 2-4 mg/kg IV are also given. The temperature of was normal after two days of treatments. Nasal discharge was completely stopped after 3 days of treatments. The local treatment of abscess was done surgically and regular dressing had been carried out. Guss, *et. al.,* 2009 reported an effective multi-component recombinant vaccine for the protection of horses from *Streptococcus equi* infection

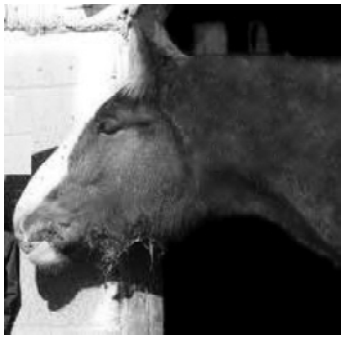

Fig. 1. Photograph showing strangle in horse

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Growth, Survival and Overall Economics of Pond Polyculture Operations with Indian Major Carps Under Differential Diet Regimen Utilizing Local Food Products in Cooch Behar District of West Bengal, India

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ABSTRACT

Two similar feeding experiments were conducted from April'2008 to February'2009 and again from April'2009 to March'2010 at outdoor farmer's ponds in Cooch Behar district of West Bengal, India, where Indian Major Carps (IMCs) namely, rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhina mrigala*) were stocked at a ratio of 3:3:4 and cultured under four different diet regimen. The fish were fed with a mixture of: fish meal, mustard oil cake, rice bran and broken rice (Treatment A); mustard oil cake, bakery wastes (unused bread) and rice meal (Treatment B); wheat flour, maize and silkworm pupae (Treatment C); and the commonly used mustard oil cake and rice bran (Treatment D or control). Growth increment was highest in Treatment C for all the fish species during both the culture periods. Survival and specific growth rates were similar for each species during both the culture periods. The food conversion ratio was better in Treatment B for all the fish species during both the culture periods, possibly due to its higher protein content. Economic analysis of the experiments revealed that Treatment C had the highest yields per ha (net profit), followed by Treatments B, A and D in decreasing order and this diet regime (a mixture of wheat flour, maize and silkworm pupae) could be suggested for pond polyculture operations of IMCs in Cooch Behar district.

Key words: *Indian Major Carps, Labeo rohita, Catla catla, Cirrhina mrigala, polyculture, diet, growth, economics*

To lower culture expenditure, fish farmers often utilize locally available ingredients for feeding the fish. In Cooch Behar district of West Bengal, India, feeding of Indian Major Carps (IMCs) are largely ad hoc, and differ from one production centre to another. Mustard oil cake and rice bran are the commonly used fish food in most of the standard fish farms (Chakraborty *et al*., in press) while many farmers would only use the cheapest ingredients like household food wastes to grocery wastes, etc. Our surveys throughout the district indicated that overall, fish food is formulated using different local ingredients, such as, mustard oil cake, rice bran, rice (broken), wheat flour, maize, bakery wastes, soybean meal, fish meal, silkworm pupae, mustard oil cake, etc. (personal observations).

Protein, the most expensive component in fish feeds, is required by fish for maintenance and growth, and the protein level needed for this function varies with species and culture environment (Delong *et al*., 1958). IMCs are cultured throughout India with average protein content in the feed of around 20% to 30%. In Cooch Behar and throughout the northern region of West Bengal, this amount can be much lower. A mixture of wastes from grocery stores commonly used as fish food in Malda district often contain not more than 10% protein and fish cultured under such conditions depend upon natural food source such as plankton for their protein requirement (personal observations). Since most fish farmers in the area are not economically sound, it would be unwise to believe that any recommendations of utilizing highly priced, good protein source as fish food would ever be met. An experiment was carried out to formulate the best possible food for IMCs under culture conditions in Cooch Behar district using different diets incorporated with locally available ingredients. Keeping in view of the economic condition of most fish farmers, ingredients were carefully selected so that they could be rationally recommended for future usage if found suitable.

MATERIALS AND METHODS

The present experiments were conducted from April'2008 to March'2010 at outdoor farmer's ponds in four villages namely, Kalakata (subdivision: Cooch Behar), Gitalda (subdivision: Dinhata), Matalhat (subdivision: Dinhata) and Maruganj (subdivision: Tufanganj) (located between latitudes: $26^0$12' N and $26^0$16' N; longitude: $89^0$44' E) of Cooch Behar district. There were total eight ponds for four treatments and the results presented in the paper are averages of two ponds for each treatment. For practical reasons, the water area in each pond were not exactly similar (it is very difficult to obtain exactly similar experimental ponds in the field); however, the areas for each of the duplicate ponds for each treatment were similar. The pond area for each treatment were: Treatment A: 0.27 ha; Treatment B: 1 ha; Treatment C: 0.60 ha; and Treatment D: 0.33 ha.

Fry of IMCs, namely, rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhina mrigala*) were stocked at a ratio of 3:3:4. The stocking density was 6000 fry/ha. Details of the feed applied are presented in Table 1 and 2. Food ingredients were manipulated from what farmers generally use or could

use (available in the locality). Although care was taken to apply food at 5% body weight of stocked fish daily, the actual dose applied could have differed in different ponds and on different days due to the apparent difficulties of management while experimenting on a large scale, and the fact that the exact weight of representing growing fish species could only be taken once in a month and daily calculations were based on approximate propositions. Fish were cultured for one year duration twice; i.e., during the 2008-2009 culture period and again during the 2009-2010 culture period. Fry were released at the start of the experiment (April'2008) and again during April'2009. Fish were harvested once in Feb'2009 (after the first culture period) and again in March'2010 (after the final culture period). Since the same ponds were being used, a one month gap was left during March 2009 between the two culture experiments and some debris were removed from the pond bottom. Among pond management, monthly netting activities were carried out to stir portions of the pond bottom and release unwanted gas. The other management practiced was addition of lime three times each year in every pond at more or less similar quantities for each treatment (92.5 – 147.5 kg/pond/year).

The fish were analyzed for the following growth parameters according to or suitably modified from Ricker, 1975 and Shokho, *et al*., 2011.

(a) Growth increment: (final weight – initial weight).

(b) Survival rate: Differences between the number of fish stocked and the number of fish at harvest would be used to calculate percent mortality in each treatment.

(c) Specific Growth Rate (SGR): $SGR = 100 [(\ln W_t - \ln W_0)/t]$; where, W_0 and W_t are the initial and final live weight of the fish (g), respectively, and (t) is culture period in days.

(d) Food Conversion Ratio (FCR): FCR = diet fed (g) / weight gain (g) of the fish.

(e) Gross fish yield (GFY): total fish harvested (kg)

(f) Net fish yield (NFY): total fish harvested (kg) – total fish stocked (kg)

(g) Net annualized production (NAP): NFY X 365 / pond surface area (ha) X growth period (days).

The overall economics of the culture operations was also calculated.

RESULTS AND DISCUSSION

The growth performance of IMCs under different diet regimen during the two culture periods are presented in Table 3. Since the ponds of different treatments were of different surface area / size, comparing some of the results (like gross fish yield, etc.) could be misleading. Hence, only some of the parameters have been chosen for further discussion. Growth increment was highest in Treatment C for all the fish species during both the culture periods (Table 3). Survival and specific growth rates were similar for each species during both the culture periods (Table 3). The food conversion ratio was better in Treatment B for all the fish species during both the culture periods (Table 3).

Gross and net fish yields were highest in Treatment B, followed by Treatments C, D and A. However, such results were only obvious since the ponds for Treatment B were largest (1 ha) and as such allowed for more stocking followed by the ponds for Treatments C (0.60 ha), D (0.33 ha) and A

Table 1. Ingredient proportion of feed.

Year: 2008-09				
Feed Ingredients (%)	**Food 1 (Treatment A)**	**Food 2 (Treatment B)**	**Food 3 (Treatment C)**	**Food 4 (Treatment D)**
Fish meal	7.23	0	0	0
Mustard oil cake	22.56	26.55	0	52.90
Rice bran	36.76	0	0	47.10
Rice (broken)	33.45	0	0	0
Wheat flour	0	0	43.67	0
Maize	0	0	42.07	0
Bakery wastes (unused breads)	0	28.29	0	0
Soybean meal	0	45.15	0	0
Silkworm pupae	0	0	14.26	0
Year: 2009-10				
Feed Ingredients (%)	**Food 1 (Treatment A)**	**Food 2 (Treatment B)**	**Food 3 (Treatment C)**	**Food 4 (Treatment D)**
Fish meal	7.19	0	0	0
Mustard oil cake	22.54	25.57	0	51.22
Rice bran	36.61	0	0	48.78
Rice (broken)	33.65	0	0	0
Wheat flour	0	0	43.88	0
Maize	0	0	42.45	0
Bakery wastes (unused breads)	0	25.48	0	0
Soybean meal	0	49.95	0	0
Silkworm pupae	0	0	13.67	0

Table 2. Proximate composition of feed.

Year: 2008-09				
Proximate composition (%)	**Food 1 (Treatment A)**	**Food 2 (Treatment B)**	**Food 3 (Treatment C)**	**Food 4 (Treatment D)**
Crude Protein	16.28	30.27	18.03	21.08
Crude Fibre	8.03	4.52	2.14	7.00
Crude Fat	9.26	3.73	4.53	7.88
Year: 2009-10				
Proximate composition (%)	**Food 1 (Treatment A)**	**Food 2 (Treatment B)**	**Food 3 (Treatment C)**	**Food 4 (Treatment D)**
Crude Protein	16.27	31.93	17.72	20.74
Crude Fibre	8.03	4.55	2.14	7.00
Crude Fat	9.25	3.56	5.48	7.95

Table 3. Growth performance of IMCs under different diet regimen during the two culture periods. (Mean values are presented).

Growth and production parameters		Treatment A	Treatment B	Treatment C	Treatment D
		Year: 2008-09			
Initial weight (g)	*L. rohita*	6.0	4.9	5.0	5.1
	C. catla	18.6	17.5	17.8	17.4
	C. mrigala	7.2	5.3	5.4	5.5
Final weight (g)	*L. rohita*	684.0	813.0	1032.0	745.0
	C. catla	1092.0	1356.0	1812.0	1022.0
	C. mrigala	624.0	792.0	949.0	682.0
Growth increment (g)	*L. rohita*	678.0	808.1	1027.0	739.9
	C. catla	1073.4	1338.6	1794.2	1004.6
	C. mrigala	616.8	786.5	943.6	676.5
Survival rate (%)	*L. rohita*	90.0	90.3	90.0	89.5
	C. catla	90.1	92.2	90.2	90.0
	C. mrigala	85.0	90.6	92.0	87.8
Specific Growth Rate (%)	*L. rohita*	1.72	1.68	1.78	1.79
	C. catla	1.47	1.43	1.54	1.46
	C. mrigala	1.61	1.65	1.72	1.68
Food Conversion Ratio	*L. rohita*	5.59	1.30	1.90	2.96
	C. catla	3.53	0.78	1.09	2.18
	C. mrigala	6.15	1.34	2.07	3.24
Gross fish yield (kg)		1167.36	5224.50	3717.38	1431.58
Net fish yield (kg)		1150.38	5171.16	3687.29	1413.68
Net annualized production		5634.56	6229.29	7477.00	5610.88
		Year: 2009-10			
Initial weight (g)	*L. rohita*	4.8	4.0	5.0	5.2
	C. catla	14.2	12.2	14.1	15.0
	C. mrigala	6.0	4.6	4.4	5.4
Final weight (g)	*L. rohita*	972.0	1024.0	1425.0	827.0
	C. catla	1720.0	1858.0	2024.0	1322.0
	C. mrigala	813.0	928.0	1228.0	735.0
Growth increment (g)	*L. rohita*	967.2	1020.0	1420.0	821.8
	C. catla	1705.8	1845.8	2009.9	1307.0
	C. mrigala	807.0	923.4	1223.6	729.6
Survival rate (%)	*L. rohita*	91.2	90.3	88.9	89.4
	C. catla	88.8	88.7	89.3	90.1
	C. mrigala	84.2	89.0	93.2	87.8
Specific Growth Rate (%)	*L. rohita*	1.76	1.84	1.83	1.67
	C. catla	1.59	1.67	1.60	1.47
	C. mrigala	1.63	1.76	1.83	1.62
Food Conversion Ratio	*L. rohita*	3.45	1.13	1.42	3.39
	C. catla	1.96	0.62	1.01	2.13
	C. mrigala	4.14	1.25	1.65	3.82
Gross fish yield (kg)		1691.07	6673.32	4639.10	1674.96
Net fish yield (kg)		1677.67	6633.12	4614.06	1658.52
Net annualized production		7534.75	8043.48	9083.77	6054.19

(0.27 ha). The net annualized production (NAP) evidently provided better opinion where Treatment C appeared far better followed by Treatments B, A and D in reducing order (Table 3). Interestingly, Treatment D contained a mixture of mustard oil cake and rice bran that is commonly used in most of the fish farms of Cooch Behar district (Chakraborty *et al.*, in press).

Rice bran and mustard oil cake have also been applied as feed in different aquaculture experiments (Azim *et al*., 2002; Dhawan and Kaur, 2002; Chakraborty *et al*., 2003).

In aquaculture, the feed forms most expensive input and accounts to 57-87 % of the total recurring expenditure (Nandeesha, 1993). In our experiment, food accounted to 57.96% and 54.43% (Treatment A), 41.01% and 41.44% (Treatment B), 39.71% and 39.57% (Treatment C) and 52.46% and 61.93% (Treatment D) during the two culture periods. The feed ingredients and their proportions were selected judiciously so that the costs could be reduced. However, this resulted in reduction in proximate composition of crude protein in all the treatments (Table 2). That the fish could overcome this could be due to the natural food like plankton (with high protein content) that are also available in most aquaculture ponds in this region (Jha, *et al*., 2003; Das, *et al*., 2011). Food remnants that are not taken by the fish, particularly the remnants of different oil cakes, and leaf litter that accumulate in most of the open ponds act as manure that aid in plankton growth. Application of mustard oil cake as manure to assist plankton production in aquaculture units has been reported by many authors including Jana and Pal, 1983, Chakrabarti and Jana, 1998, Srivastava, *et al*., 2006, etc. Better food conversion ratio value in Treatment B could be due to its higher protein content that was easily converted to flesh.

Economic analysis of fish culture operations are presented in Table 4. Treatment B showed the biggest margin of gross profit in both the culture periods since the ponds were large and more fish could be harvested and sold. However, the net profit showed that Treatment C had the highest yields per ha, followed by Treatments B, A and D in decreasing order (Table 4). Since the other culture parameters were similar in all the treatments, any differences in fish growth and yields could only have resulted due to diet differences. As such, the food regime (Treatment C) containing a mixture of wheat flour, maize (about 42 – 44% each) and silkworm pupae (about 13 – 14%) should the best growth results. It should be pointed out that Treatment C had only about 17.72 – 18.03% of crude protein, that was less that Treatments B (> 30%) and D (> 20%) (Table 2).

According to Manjappa, *et al*., 2002, part of dietary protein may be utilized (oxidized) as an energy source, if the

Table 4. Economic analysis of fish culture operations under different diet regimen during the two culture periods.

	Year: 2008-09			
	Treatment A	**Treatment B**	**Treatment C**	**Treatment D**
Expenditure				
Food	Rs. 24727.00	Rs. 43437.50	Rs. 32720.00	Rs. 22916.00
Fish fry	Rs. 1950.00	Rs. 6650.00	Rs. 3950.00	Rs. 2500.00
Lime	Rs. 880.00	Rs. 680.00	Rs. 1120.00	Rs. 720.00
Netting	Rs. 3600.00	Rs. 3600.00	Rs. 3600.00	Rs. 3600.00
Transport (Rs. 3/ kg)	Rs. 3502.00	Rs. 15673.50	Rs. 11152.00	Rs. 4285.50
Middleman/ wholesaler commission (6% of total sale)	Rs. 8006.50	Rs. 35884.00	Rs. 29857.00	Rs. 9657.50
TOTAL	Rs. 42665.50	Rs. 105925.00	Rs. 82399.00	Rs. 43679.00
Income				
Sale of fish	Rs. 133442.50	Rs. 598071.50	Rs. 497622.00	Rs. 160964.00
Profit				
Gross profit / season	Rs. 90777.00	Rs. 492146.50	Rs. 415223.00	Rs. 117285.00
Net profit / ha / season	Rs. 336211.00	Rs. 492146.50	Rs. 692038.00	Rs. 335409.00
	Year: 2009-10			
	Treatment A	**Treatment B**	**Treatment C**	**Treatment D**
Expenditure				
Food	Rs. 35072.00	Rs. 73269.50	Rs. 48403.00	Rs. 42694.50
Fish fry	Rs. 2200.00	Rs. 6950.00	Rs. 4200.00	Rs. 2800.00
Lime	Rs. 850.00	Rs. 850.00	Rs. 1317.50	Rs. 807.50
Netting	Rs. 3600.00	Rs. 3600.00	Rs. 3600.00	Rs. 3600.00
Transport (Rs. 3/ kg)	Rs. 5073.00	Rs. 20019.50	Rs. 13926.00	Rs. 5024.50
Middleman/ wholesaler commission (6% of total sale)	Rs. 17637.00	Rs. 72106.00	Rs. 50866.50	Rs. 14011.50
TOTAL	Rs. 64432.00	Rs. 176795.00	Rs. 122313.00	Rs. 68938.00
Income				
Sale of fish	Rs. 293955.00	Rs. 1201770.00	Rs. 847775.00	Rs. 233532.50
Profit				
Gross profit / season	Rs. 229523.00	Rs. 1024975.00	Rs. 725462.00	Rs. 164594.50
Net profit / ha / season	Rs. 850085.00	Rs. 1024975.00	Rs. 1209103.00	Rs. 498771.00

*Values rounded off to the nearest Rs. 0.50. Labour charges are included under netting charges. The pond owners did the other works like daily food administration, and collection and disposal of dead fish from the pond, etc. (as such, there wages were not considered for calculation).

diet is deficient in non-protein energy. Besides, unused food rich in protein can severely increase the nutrient level of the bottom soil and water quality of the pond, thus affecting fish growth and survival. From the results obtained from water and soil quality analysis, Treatment C appeared to be the most suitable for a majority of the parameters (Chakraborty *et al*., in press). Adult IMCs require 30% dietary protein for proper growth and survival, while fry and fingerlings require 40 and 35% dietary protein, respectively (Murthy, 2003). IMCs, like other fish, do not have an absolute requirement for protein, but need a balanced mixture of amino acids (Murthy, 2003). Silkworm larvae protein isolate, particularly the 5th larval instar is a high quality protein source with a well-balanced composition of essential amino acids (Wu *et al*., 2011). This could explain for the fact that Treatment C resulted in better growth compared to Treatment B despite having lower crude protein content.

From the present experiment, Treatment C (a mixture of wheat flour, maize and silkworm pupae) could be suggested for pond polyculture operations of IMCs in Cooch Behar district. According to recommendations by Chow, 1982, development of practical diets for IMCs should be based on ingredients which are inexpensive and easily obtainable. Silkworm pupae are available in Cooch Behar district since they are cultured here Pandey, *et al*., 2010. Treatment B, with a mixture of mustard oil cake, bakery wastes (unused breads) and soybean meal also yielded good results. The existing practice of applying a mixture of mustard oil cake and rice bran (Treatment D) did not give encouraging results and farmers should be recommended to change such feeding regime in future. However, more research is needed before a balanced and cost effective diet is finally optimized for the pond conditions in Cooch Behar district.

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Two Undescribed Species of *Pseudocercospora* Speg. from India

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ABSTRACT

This communication deales with the descriptions and illustrations of undescribed species of fungus genus *Pseudocercospora viz; P.casearíae* Singh and Mall sp. nov. on *Casearia tomentosa* (Symadaceae) and *P. zizyphii* on *Zizyphus* sp. (Rhamnaceae) collected from North Western *Tarai* Forests of U.P. *P. Casearíae* is characterized by infection spots hypogenous, subcircular to circulare, upto 5 mm in diam in descrete patches in begning becoming effuse later on. Colonies effuse, tufted mid to dark brown. Mycelium immersed. Stromata present, fascicles 27-37mm in diam. Conidiophores macronematous, synnematous, individual threads unbranched, generally flexuous, often narrow cylindrical adpressed near the base, somewhat towards the apex, olivaceous brown to light brown, smooth, 18-37 x 5-6 mm in diam. Conidiogenous cells integrated, terminal, often monoblastic and percurrent becoming polyblastic later on; sympodial and denticulate broad conidial denticles and no scars or thin scars. Conidia solitary, simple acrogenous on young conidiophores becoming acropleugenous later on, mostly cylindrical smooth or rugulose, straight to curved, apex obtuse, base obconico-cylindrical with numerous transverse septa, 29-68 x 3.5-5 mm in diam, hilum unthickened. *P. zizyphii* in characterized by infection spots amphigenous, subcircular to circular , upto 7 mm in diam; in desrete pathches in beginning becoming effuse later on. Colonies effuse, tufted mid to dark brown. Mycelium immersed. Stromata present, fascicles upto 37 mm in diam. Coniodiophors macronematous, synnematous individual threads unbranched generally flexuous often narrow. Conidiogenous cells integrated, terminal often monoblastic, rarely polyblastic, sympodial scars thin. Conidia solitary, rarely catenate, simple on young acropleurogenous, mostly cylindrical, smooth, apex obtuse, base obconico-cylindrical with numerous transverse eusepta (generally) (3-9), 13-77x 3-5 mm in diam; hilum unthickened.

Key words: *Foliicolous fungi, Pseudocercospora, species novel*

On systematic and periodic survey of North Western *Tarai* Forest of U.P. a number of collections of living leaves exhibiting leaves spots and blights were encountered of these, upon critical examination and comparison of morphotaxonomic feature with those of the allied forms two taxa of species rank have found to be hitherto undescribed. These are described and illustrated as *Pseudocercospora casearíae* Singh and Mall sp. nov. on *Casearia tomentosa* (Symadaceae) and *P. zizyphii* Singh and Mall sp. nov. on *Zizyphus* sp. (Rhamnaceae).

MATERIALS AND METHODS

During collection trips infected leaf samples were taken in separate polythene bags from North Western Tarai Forest of Uttar Pradesh. Suitable mounts of surface scrapping and free hand cut sections were prepared from infected portions of the leaf samples. Microscopic slides were prepared in cotton- blue lactophenol mixture, slides were examined and camera lucida drawing were made. Morphotaxonomic determinations of taxa were done with the help of current literature and resident expertise available. Holotypes have been deposited in HCIO, IARI, New Delhi and Isotype retained in the departmental herbarium for further reference.

RESULTS AND DISCUSSION

Taxonomic Description

***Pseudocercospora casearíae* sp. nov. (Fig. 01)**

Maculae hypogenae, sub circulares vel circulares, usque 5 mm in diam, inceptio in fragmenti posterius effusae. Coloniae effusae, brunnae vel atrobrunnae. Mycelium immersum. Stromata presentia, fascicles 27-37mm in diam. Conidiophora macronematosa, synnematosa, non-ramosa, plerumque flexuosa, cylindrica, adpressae ad basim, inflati ad apicem, olivaceo brunnea vel pallide brunnea, laevia, 18-37 x 5-6 mm in diam. Cellulae conidigenae in conidiophoris integrate, terminales, plerumque monoblasticae et percurrentae, polyblasticae, posterius, sympodialae et denticulatae. latae denticulate, non cicatrices vel cicatrices non incerassata. Conidia solitaria, simplicia, acrogena ad conidiogena, acropleurogenosa posterius, plerumque cylindrica, laevia vel rugulosa, recta vel curvata, apicem obtusa, basim abconicocylindrica cum numerosa transverse septata, 29-68 x 3.5-5 mm in diam, hila non-incrassata.

In foliis vivis *Casearia tomentosa* Linn. (Samydaceae), Katarniaghat Wildlife Sanctuary, Bahraich (U.P.) India, 13th Jan; 2007, leg., D.P. Singh, BRH-1,579, DPS-0,179 (Isotypus), HCIO - 48,519 (Holotypus).

On living leaves of *Casearia tomentosa* Linn. (Samydaceae), Katarniaghat Wildlife Sanctuary, Bahraich (U.P.) India, 13th Jan; 2007; leg; D.P. Singh, BRH-1,578, DPS-0,178 (Isotype), HCIO -48,519 (Holotype).

Survey of Literature indicates that there is no record of *Pseudocercospora* species of this type on the host family. Therefore, it is described and illustrated as a new species to acommodate it.

***Pseudocercospora zizyphii* sp. nov. (Fig. 02)**

Maculae amphigenae, sub circulares vel circulares,

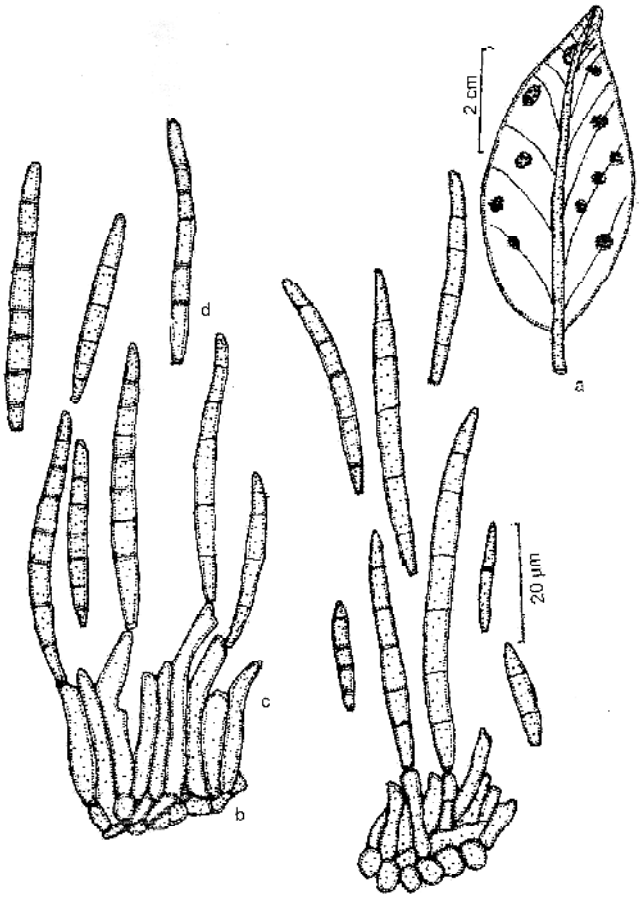

Fig. 1: *Pseudocercospora caseariae* Singh and Mall sp. nov. a. Infected leaf, b. Stroma, c. Conidiophores, d. Conidia

usque 7 mm in diam, inceptio in fragmenti posterius effusae. Coloniae effusae, brunnae vel atrobrunnae. Mycelium immersum. Stromata presentia, fascicles 37 mm in diam. Conidiophora macronematosa, synnematosa, non-ramosa, plerumque flexuosa, cylindrica, adpressae ad basim, inflati ad apicem, olivaceo brunnea vel pallide brunnea. Cellulae conidigenae in conidiophoris integrate, terminales, plerumque monoblasticae et percurrentae, polyblasticae, posterius, sympodialae et denticulatae. latae denticulate, non cicatrices vel cicatrices non incerassata. Conidia solitaria, simplicia, acrogena ad conidiogena, acropleurogenosa posterius, plerumque cylindrica, laevia vel rugulosa, recta vel curvata, apicem obtusa, basim abconico cylindrica cum numerosa transverse euseptata, (3-9) 13-77 x 3-5 mm in diam, hila non-incrassata.

In foliis vivis *Zizyphus* sp. Juss. (Rhamnaceae), Katarniaghat Wildlife Sanctuary, Bahraich (U.P.) India, 13th Jan; 2007, leg; D.P. Singh, BRH-1,583, DPS-0,183 (Isotypus), HCIO - 48,522 (Holotypus).

On living leaves of *Zizyphus* sp. Juss. (Rhamnaceae), Katarniaghat Wildlife Sanctuary, Bahraich (U.P.) India, 13th Jan; 2007, leg; D.P. Singh, BRH-1,583, DPS-0,183 (Isotype), HCIO - 48,522 (Holotype).

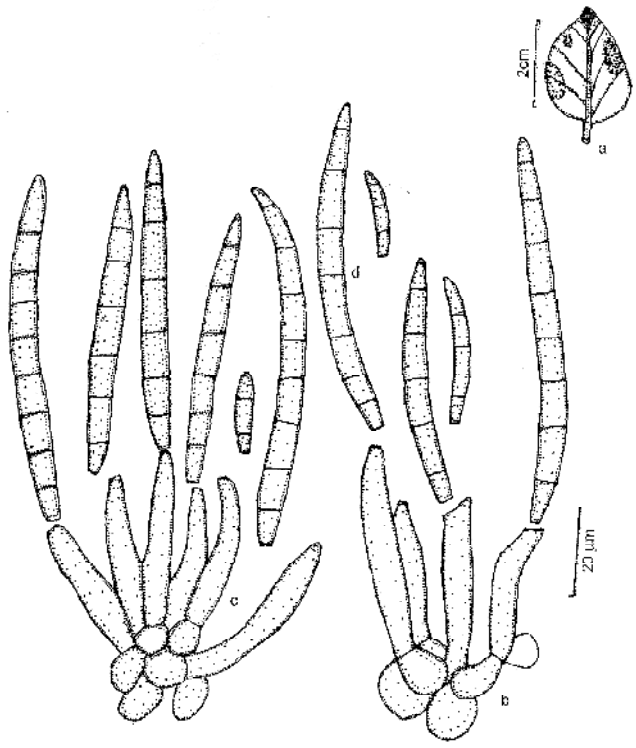

Fig. 2: *Pseudocercospora zizyphii* Singh and Mall sp. nov. a. Infected leaf, b. Stroma, c. Conidiophores, d. Conidia

This type of fungus has not ever been reported on this host genus. Therefore, it is described and illustrated as a new taxon of species rank.

The perusal of literatures (Bilgraami, *et.al;* 1979, 1981, 1991; Ellis, 1971, 1976; Jamaluddin, *et al.,* 2004; Meenu, *et al.,* 1997, Meenu and Kamal, 1998; Sarbhoy, *et al.,* 1986, 1996; Singh and Mall, 2007a, 2007b, 2008) reveals that both the fungal taxon has not been reported either from North Western Tarai Forests of U.P. or India. Therefore, both or new record to Indian mycoflora.

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Isolation and Identification of Fungal Species from Bio-compost

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ABSTRACT

Bio-compost, widely used to produce natural organic fertilizers, is a mixture of decayed organic materials. Bio-compost can be found in a warm, moist, aerobic and anaerobic environment. The proper use of bio-compost in the agricultural fields is required for beneficial growth of plants, which is needed for humans as well as environment. Previous studies demonstrated a high number of bacteria, which could be detrimental to the environment. The present study was designed to determine the fungal status. Fifteen samples from different sites were assessed. Results showed that *Aspergillus* sp., *Saccharomyces* sp., *Candida.* sp. and different types of yeasts and molds predominated in all samples. All the isolates were observed under light microscope and biochemical test was performed for further identification.

Key words *Biocompost, arganicmatter, Aspergillus, Condide, Carbon*

Compost is a mixture of decayed organic materials decomposed by microorganism in a warm, moist, and aerobic environment, releasing nutrients into readily available forms for plant use. Bio-compost includes the animal wastes such as cattle dung, buffalo manure, poultry wastes, rural and urban organic wastes, and also human wastes can be used for bioconversion as organic manure.

The high organic matter content and biological activity make composts effective in a variety of applications, including erosion control, revegetation, biofiltration and bioremediation (Alexander, 1999).

Fungi possess the ability to growing bio-compost. Therefore, in the present investigation an attempt has been made to isolate and identification of fungi from different substrates like underground coal nesting material of birds, animal and municipal refuse, decomposition of plant materials, mushroom compost, vermi compost, poultry litter and tobacco products.

Fig. 1. Collecting bio-compost from decomposing area.

The Bio-Compost samples contained valuable amounts of major plant nutrients (*i.e.* nitrogen, phosphate, potash, sulphur and magnesium). At the maximum permitted organic material application rate to agricultural soils (*i.e.* 250 kg total N/ha/annum) in Nitrate Vulnerable Zones-NVZs, the Bio-compost typically supplies *c.*140 kg/ha phosphate, *c.*70 kg/ha potash, *c.*160 kg/ha magnesium and *c.*230 kg/ha sulphur. The readily available N content of the Bio-compost was equal to 16% of the total N content and would be regarded as a low readily available N organic material (<30% of total N) and not subject to closed spreading periods in NVZs.The Bio-compost also provides a valuable source of stable organic matter (typically 5-7 t/ha), which will in the long-term improve soil physical (*e.g.* drainage, water infiltration rates, plant available water supply *etc.*) and biological properties (*e.g.* soil microbial biomass size and activity).

Table 1. Composition of bio-compost.

Constituents	% Quantity
Organic matter	61.20
Organic carbon	35.5
Total nitrogen	1.95
C/N	18.20
Phosphorus	1.35
Potassium	0.88
pH	7.67
Ash content	18.20

Bio-compost is used to produce natural organic fertilizers, production of food and feed supplements for cattle and poultry feed stocks, production of plant protoplast for genetic manipulation, preparations of pharmaceuticals, baking, malting and brewing, extraction of fruit juices and processing of vegetables, botanical extraction for maximum oil yield, processing of starch and fermenting tea and coffee.

MATERIALS AND METHODS

The complete research was conducted at the Microbiology Laboratory of Stamford University Bangladesh. A total of 15 samples were collected from different sites.

The following precautions were carefully taken during collection of the samples:-

The samples were collected in sterile plastic containers. The samples were transported to the laboratory as promptly as possible after collection. Standard microbiological practices were maintained during the sampling time.

Samples collected from different sites were tested to determine their pH values. The conventional strip method (supplier) was employed to determine the pH.

Prior to the inoculation of fungi, serial dilution was carried out. At first, 10 mg of each sample was added to 90 ml of sterile normal saline (0.88% Nacl). It was considered as the fast sample. Then 1 ml from each of the sample was transferred to 9 ml of normal saline to make the 10^{-1} dilution. This particular step was repeated till the 10-5 dilution.

By using sterile tips, 0.1 ml from the 10^{-3}, 10^{-4} and 10^{-5} dilutions of each sample was transferred on the SDA plates and spread plate method was employed. All the plates were incubated at 25^{0}C for 24-72 hours.

All SDA plates were incubated at 25^{o}C for 72 hours for the growth of fungus. Sabouraud Dextrose Agar medium, pH adjusted to 6.5, was used for the isolation of fungus. SDA medium is also used for cultural characterization of the fungi. After cooling (45^{0}C), approximately 15 ml of medium was poured into sterilized Petri plates inside a laminar flow cabinet and allowed to solidify. In case of slant preparation, approximately 2ml of the agar medium was poured into screw cap vials and sterilized and kept at room temperature for solidification. SDA medium was used for cell mass production and prepared by the addition of Dextrose.

The standard growth was used in all experiments was SDA. Petriplates were incubated by spreading of the inoculums and transferred to incubator for different condition like temperature, pH and time. All the fungi were examined and characterized depending on size, shape and reproducing system and other morphological characteristics of fungi under the light microscope.

RESULTS AND DISCUSSION

Characteristics of the isolates

The morphological and cultural characteristics of different strains isolates from bio-compost vary each other. These characteristics have great effect on the isolation and identification of yeast and mold separately. A comparative preliminary investigation on some of these important characteristics of the two isolates was carried out.

Cultural characteristics

The fungus was grown on SDA agar plates at 25^{0}C for 72 hours of studying their colonial characteristics (size, shape, color, margins, elevation, optical characteristics, etc. A comparative result on the basis of the characteristics of the strains is shown Table.

Table 2. Colonial morphology of the Fungi isolated from bio-compost.

Sample No.	Size	Color	Shape	Appearance	Elevation	Margins	Opacity
A.	Large	Yellow	Circular	Gummy	Raised	Entire	Opaque
B.	Small	Yellow	Circular	Gummy	Slightly Raised	Entire	Opaque
C.	Large	White	Irregular	Cottony	Convex	Filamentous	Opaque
D.	Moderate	Black Centered Colony	Irregular	Cottony	Convex	Undulate	Opaque
E.	Moderate	Yellow	Circular	Gummy	Raised	Entire	Opaque
F.	large	Pinkish White	Circular	Gummy	Convex	Entire	Opaque
G.	Small	Yellow	Circular	gummy	Raised	Entire	Opaque

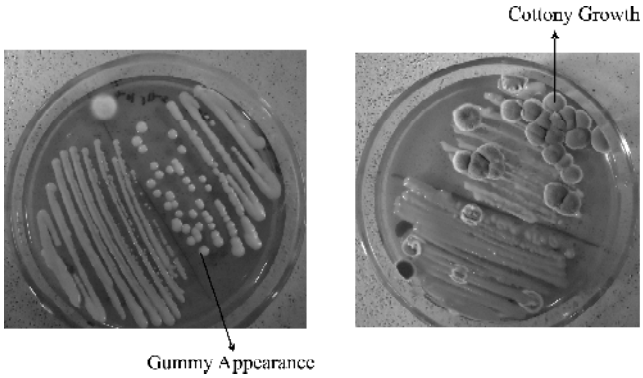

Fig. 2. Colonial morphology of the Fungi isolated from bio-compost

Morphological characteristics

The young of fifteen isolates were grown in SDA at 25^{0}C for 72 hours. The morphological characteristics of these cells such as shape, spores, reproducing system, etc. were observed under the light microscope. Some important characteristics of these isolates are presented below the table.

Table 3. Morphological characteristics of the isolates yeast

Characteristics	Sample A	Sample B	Sample E	Sample F	Sample J	Sample M
Shape	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid
Reproducing system	Budding	Budding	Budding	Budding	Budding	Budding
Spores	Ascus with two ascosphore	Ascus with one ascosphore	Ascus with one ascosphore	Ascus with one ascosphore	Ascus with with one ascosphore	Ascuc with two ascosphore

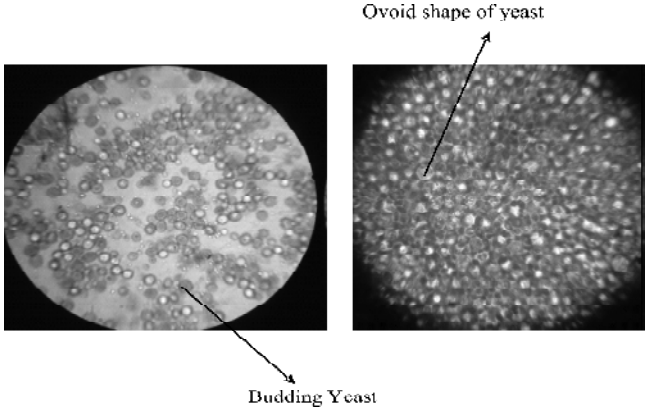

Fig. 3. Microscopic appearance of yeasts.

Biochemical Test of Yeast:

Identification of Mold

Among the fifteen samples the young of fifteen isolates were grown in SDA at 250c for 72 hours. The morphological

Table 4. Carbohydrate Fermentation by different types of yeasts.

Sample no.	Media						Suspected organisms
	Dextrose phenol broth		Sucrose phenol broth		Lactose phenol broth		
	Color	Gas production	Color	Gas production	Color	Gas production	
A.	Yellow	+	Yellow	+	Red	-	*Candida.sp*
B.	Yelow	+	Yellow	+	Yellow	+	*Saccharomyces.sp*
E.	Yellow	+	Yellow	+	Yellow	+	*Saccharomyces.sp*
F.	Yellow	+	Yellow	-	Yellow	+	Saccharomyces.sp
J	yellow	-	Red	-	Red	-	Candida.sp
M	Yellow	+	Yellow	-	Red	-	Candida.sp

characteristics of these cells such as shape, spore, mycelium, conidia, etc. were observed under the light microscope and identified the isolated type of molds which is presented below the table.

Generally bio-compost consists of animal feces, cattle dung, poultry wastes, rural and urban organic wastes, and also human excreta. Bio-compost is high in nitrogen, a main ingredient in commercial fertilizer. Bio-Compost plays various beneficial roles in agricultural fields and also other environment. It improves the physical and biological properties of soils. In addition, some types of compost also suppress soil borne plant pathogens.

Several mechanisms are involved, however, many studies have been developed to identify the compost microbiota able to reduce the activity of plant pathogens and additionally, able to favor the plant growth by the production of phytohormones, vitamins and /or amino acids Estrella, *et al.,* 2007.

Bio-compost also contains different types of pathogenic microbes. This has many detrimental roles for human health and also some plants. In most of the cases, during the preparation of bio-compost the environment can be polluted by the involvement of hazardous materials also some wide variety of pathogenic microbes.

Bio-compost may consist of different types of microorganisms like bacteria, fungi and protozoa. Bacteria including fecal coliform, fecal *Streptococci, Staphylococci, pseudomonas, Clostridium* and some enteric bacteria which have many beneficial and detrimental roles in bio-compost. Fungus and protozoa can also decompose microorganisms found in bio-compost.

A few studies as well as the ongoing study in our laboratory demonstrated a high number of bacteria, in bio-composts or in the candidate fertilizers which could be detrimental to the environment and crop productivity as well.

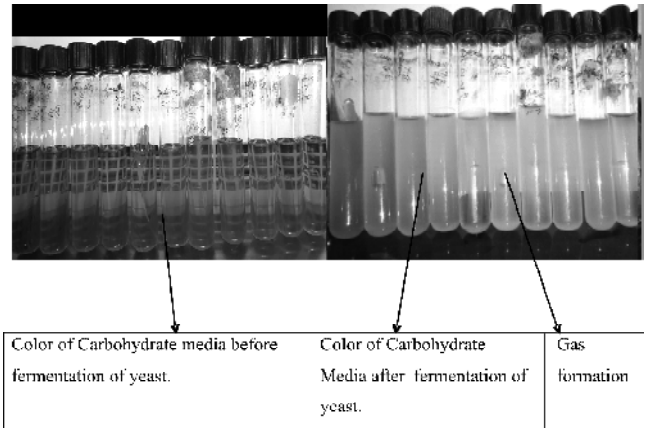

Fig. 4. Following figures indicate biochemical test of yeast.

Table 5. Identification of mold from bio-compost.

Sample No.	Colonial Morphology	Microscopic appearance	Isolated types
C.	Rapidly growing white, cottony fungus warm over the entire plate, white colonies Become black as a culture matures.	Singled cell spores (conidia), the vesicle; septate Mycelium,	*Aspergillus.* spp.
H.	Rapidly growing white, cottony fungus warm over the entire plate, white colonies Become black as a culture matures.	Singled cell spores (conidia), the vesicle; septate Mycelium,	*Aspergillus.* spp.
K	Rapidly growing black, cottony fungus warm over the entire plate, Black colonies Become Brown as a culture matures.	Singled cell spores (conidia), the vesicle; septate Mycelium,	*Aspergillus.* spp.

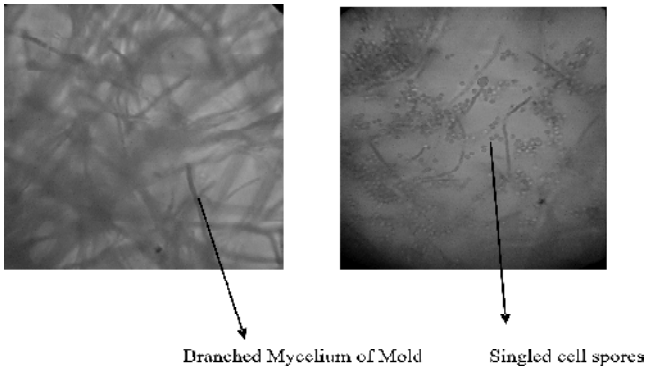

Fig. 5. Microscopic observation of mold

Different types of bacteria, protozoa and some fungal species were found in bio-compost. This led me to focus on the fungal study from the similar condition.

The present study was designed to determine the fungal status. An extensive screening procedure has been performed to isolate suitable fungi from various sources. Initially 15 isolates were obtained, purified and selected for isolation and identification of fungi. On the basis of growth characteristics, different isolates were selected for final study and these were identified as *Aspergillus* sp., *Saccharomyces* sp., *Candida* sp. and some undefined Mold and yeast. A comparative evaluation was made among those isolates to find out indigenous fungi which would fulfill all desirable criteria of bio-compost fungi.

Water iodine and Methylene blue solutions are wonderful dye for microscopic observation of yeast were used as staining dye and appeared ovoid shape yeast under the microscope. Different types of yeast isolates were examined. Biochemical test was performed for further conformation results of Yeast.

The microscopic and cultural characteristics of the yeasts and molds were investigated. The colonies of yeast were large, moderate in size and were filamentous, convex, undulate, on agar media. The size and shape of the cells of yeast were different than the other five mold isolates. The cells of yeast were small and both ovoid and spherical in shape whereas the cells of five were large, cottony, and filamentous in shape.

Various nutritional and environmental conditions usually affect the growth and isolation & identification is affected by initial inoculum concentration. In this study we found that the maximum isolates were yeast and the rest of the growth was identified as mold growth. The result in this study showed that the species reflected in the mold was small number when compared to the yeast. This result is consistent. Nevertheless, the additional insight could be vivid by the presence of *Aspergillus* sp. *Saccharomyces* sp., *Candida* sp. and some undefined Mold and yeast.

This data indicates that the supplied compost sample was rich in nutrient containing a supporting habitat for the growth of fungus. Fungi may play an important role in the degradation of biomass, yet their presence and participation during the initial phases of composting has been poorly characterized.

In spite of large diversity of fugal kingdom, recent finding can open new prospective and this will make fungal biology even more fascinating. In futures the complete clarification of the fungal strain can be done.

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SHORT COMMUNICATION

Effect of Concentrated Acid on the Germination Percentage of Seeds of Siris (*Albizia Lebbeck*, L. Benth)

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Albizia lebbeck (L.) Benth native to tropical southern Asia, and widely cultivated and naturalized in other tropical and subtropical regions. Being one of the most widespread and common species of *Albizia* worldwide, it is often simply called "siris" though this name may refer to any locally common member. In Urdu it is called saras.

Albizia lebbeck (L.) Benth. belonging to the family mimosaceae. It is medium sized deciduous fast growing tree met with in central Indian forests. The plant parts are used for the treatment of various diseases.

Concentrated sulphuric acid, nitric acid and hydrochloric acid pretreatment were given to the freshly harvested seeds for different durations. At the end of treatment the seeds were thoroughly washed in running tap water for at least half an hour and then in distilled water and were finally kept for germination test. Chemicals have been recommended and used to overcome hard and impermeable nature by the seed coat. The chemicals are effective in softening hard seed coats and rendering them permeable to water. The method used is according Porter, *et al.,* 1974 and Tomar, *et al.,* 2011.

Seed germination responses of certain wild and cultivated plants to various chemical treatments, aimed at breaking dormancy and improving the germination percentage have been comprehensive studies (Pandeya, 1975 and Pandeya and Pathak, 1980) Table 1 shows that concentrated H_2SO_4 is more effective in seed germination of *Albizia lebbeck*. H_2SO_4 shows significant results treated with 15 seconds shows highest 95% seed germination, HCl shows 90% maximum seed germination in 5 seconds treatment. However, HNO_3 shows insignificant results.

Table 1. Effect of concentrated acid scarification on the germination percentage of seeds of *Albizia lebbeck* (L.) Benth.

S. No.	Treatment	Time (Sec.)	Germination %
1	Control	-	75
		5	80
		10	90
2	H_2SO_4	15	95
		20	70
		25	65
		5	80
		10	75
3	HNO_3	15	60
		20	54
		25	40
		5	90
		10	85
4	HCl	15	80
		20	72
		25	50

Treatment of H_2SO_4 on the seeds of *Albizia lebbeck* which were collected from different study sites, shows positive effect. Maximum germination percentage calculated in 15 sec. Treatment with H_2SO_4 95% and minimum 66% under the treatment of 25 sec. Therefore it is concluded concentrated H_2SO_4 is suitable chemical of the breaking dormancy and germination of seeds of *Albizia lebbeck*. It also confirm the previous work of Shrivastava, 1966, Kumar and Mathur, 1980 and the results are similar with the findings of Khanduri and Negi, 2010, Ramkrishnan, 1963d with *Setaria glauca*, Nayak and Mishra, 1975 with *Sida veronicifolia* and Tomar, *et al.,* 2011 with *Albizia lebbeck*.

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There is a minor topographical mistake in Table 2. in T3 treatment the cfu value should be read as 9.2×10^{6} instead of 9.2×10^{6} at 21st day.

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