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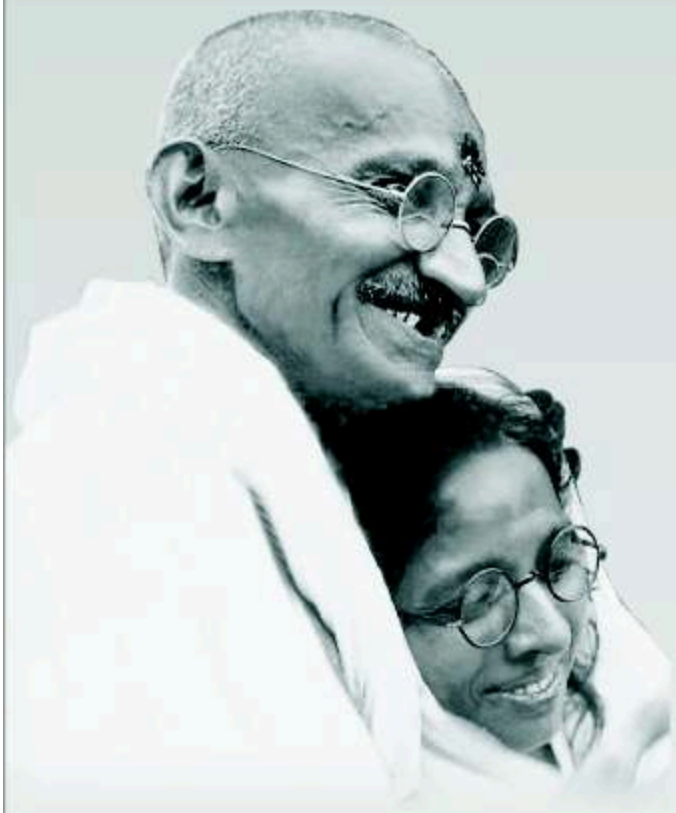

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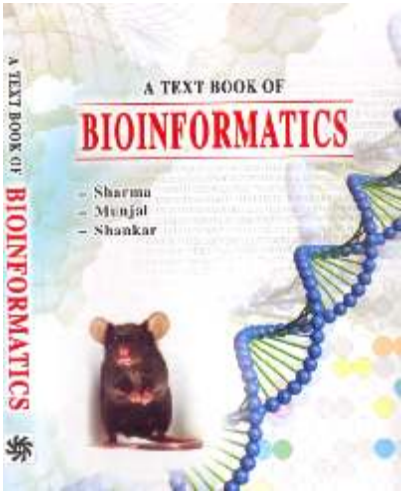
Volume 25 No. 2, 2011

Book Review

"A Textbook of Bioinformatics "

Vinay Sharma, Ashok Munjal and Asheesh Shanker
Rastogi Publications, Meerut



The book entitled "A Text Book of Bioinformatics, provides a solid foundation in basic bioinformatics and is important for PG students of Biotechnology & Bioinformatics, together with other disciplines in life sciences, where bioinformatics has been applied.

The book has been divided into thirteen chapters, which will be helpful to readers to know and grasp the basic concepts of bioinformatics. First three chapters of the book provide an easy introduction to the field of Bioinformatics, Computers and Internet technology. Chapter 4 gives a glimpse of search engines, various search strategies as well identification of selection of key words for effective searching on Internet, which has become a key feature of today's research.

Databases are the backbone of bioinformatics research. For beginners the chapter related to biological databases is very good starting point to know various databases related to nucleotide/protein sequences and biomolecular structures. The chapter related to sequences analysis deals with definition and algorithms of sequence alignment. This includes a very good explanations of Dot plot, dynamic programming and BLAST and FASTA programs for database similarity search. Moreover the chapter provides an idea of multiple sequence alignment. Phylogenetic analysis is essential to know evolutionary relationship between sequences/organisms. Various methods used to reconstruct phylogenetic relationship are nicely discussed in this chapter. Additionally tools to construct the tree give useful information. Since this is the time of high throughput research, microarray technology emerges as a technique which deals with thousands of genes/proteins in a single experiment. The chapter on microarray gives glimpse of methods to construct microarray and its data analysis, and also provides information about databases related to microarray data. Drug discovery and development is another area of active research in bioinformatics. *In silico* analysis greatly reduces the time to produce a drug. The overview of drug discovery and development gives all such information. The last chapter provides useful information of BTIS network in India. In nutshell, I highly recommend this book to the beginners in Bioinformatics as well as to those who want to acquire scientific information on specialized area of Bioinformatics.

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Antibacterial Potential of Extended Spectrum β-Lactam Antibiotics Against *M. tuberculosis*

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Abstract :

Background & Objective: The prevalence of multidrug-resistant tuberculosis (MDR-TB) with the emergence of HIV/TB co-infections is increasing throughout the world. Thus the anti-mycobacterial potential of Extended Spectrum β-lactam Antibiotics was tested to know if these antibiotics can be used as a treatment regimen against *M tuberculosis*.

Design: After biochemical and molecular biology tests, finally 233 *M. tuberculosis* positive clinical isolates out of 250 clinical isolates were used for experiments. Anti-mycobacterial activity was analyzed using disc diffusion assay. Extended Spectrum Penicillins and Broad Spectrum Cephalosporines were used alone and with clavulanic acid (CA). The appearance of Zone of inhibition was observed as the anti-tuberculosis potential of β-lactam Antibiotics.

Results: No zone of inhibition has appeared for the antibiotics, when used alone, except for ceftriaxone. When clavulanic acid (CA) was used as an inhibitor of -lactamases along with the antibiotics, several time increase in the zone of inhibition was observed. The zone of inhibition was found 18-24 mm for carbenicillin + CA, 12-15 mm for Mezocillin +CA, 10-13 mm for Piperacillin + CA, 11-14 mm for Ticracillin + CA, and 12-16 mm for Cefotaxime + CA proving Extended Spectrum -lactam Antibiotics a potential agent against *M. tuberculosis*. Broad spectrum β-lactam antibiotic like ceftriaxon also found to possess good anti-tuberculosis potential and is least affected by -lactamase enzymes.

Interpretation & Conclusion: Extended Spectrum -lactam Antibiotics possess potential anti-tuberculosis activity and thus can be used as a potential drug regimen against *Mycobacterium tuberculosis* specifically in case of HIV/TB co-infections.

Key Words: *Mycobacterium tuberculosis*, Extended Spectrum -lactam Antibiotics, Drug Susceptibility

INTRODUCTION:

Bacterial infections such as infections caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) has been the leading cause of death worldwide. According to the World Health Organization (WHO) report 2010, the global burden of tuberculosis (TB) is overwhelming and approximately 1.7 million people died due to TB in the year 2009. The conditions such as HIV/TB co-infection make the situation rather more complicated. There were recorded about 4.0 lakh deaths among people living with HIV due to TB in 2009. Further, the emergence of drug resistant tuberculosis is increasing the number of deaths. As per the WHO surveillance report 2010 on drug resistance in *M. tuberculosis*, in the year 2008, estimated 390000–510000 cases of MDRTB (Multidrug Resistant Tuberculosis) emerged globally (WHO report 2010). Although, chemotherapy is available for treating the drug resistant tuberculosis but it has its own side effects, specifically in the case of HIV/TB co-infections where the patients are immunocompromised and physiologically not in a situation to tolerate the chemotherapy for TB treatment. Thus, there is a need to look for an alternative treatment regimen for the treatment of such type of mycobacterium infections. β-lactam antibiotics have been used for treating a large number of bacterial infections, but in case of tuberculosis it has never been evaluated as a treatment strategy since mycobacteria produce β-lactamase enzymes and are resistant to β-lactam antibiotics. However, some studies showed that when used in combination with the inhibitors of β-lactamase enzymes such as clavulinic acid and sulbactum, the β-lactam antibiotics have been found to inhibit the growth of *M. tuberculosis* (Cynamon and Palam,1983; Sorg and Cynamon, 1987; Chambers *et a*l., 1998; Segura *et al*., 1998). In this study we evaluated the anti-tuberculosis effect of extended spectrum and

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broad spectrum β-lactam antibiotics on the clinical isolates collected from the clinical settings located in District Saharanpur, Uttar Pradesh, India.

MATERIALS AND METHODS

A total of 250 clinical isolates of *M. tuberculosis* were collected from different clinical settings of District Saharanpur, Uttar Pradesh, India. These clinical isolates were given the numbers from S001 to S250 for identifying them. The culture of positive strain of *M. tuberculosis* H37Rv was used as a control. The chemicals and kits used were of standard companies and the Bio-Safety Level-3 (BSL-3) labs at IFB-MRL, Saharanpur were used for the experiments on *M. tuberculosis*. The experiments were conducted at the Department of Zoology, M.S. College Saharanpur, under CCS University Meerut, U.P, India, and also outsourced as and when required from IFB-Molecular Research Laboratory, Saharanpur, U.P., during 2007-2009. The materials used and the methodologies adopted are as follows.

Primary Isolation of *M. tuberculosis*

From clinical settings located in the district Saharanpur, U.P., the microscopically positive sputum samples were collected in sterile sputum collection containers. The so collected sputum specimens were again tested in the laboratory for confirmation of the presence of *M. tuberculosis* with ZN or AFB staining as per the National Tuberculosis Institute (NTI) -Manual (1998). On observing under Binocular Microscope, the slides which showed pink rods like Acid Fast Bacilli (AFB) were marked as TB +ve and other slides as TB –ve.

Primary isolation of *M. tuberculosis* from the ZN positive clinical sputum samples was done by decontamination using Petroff's Sodium Hydroxide Method (Petroff, 1915) and then culturing the so obtained sterile and concentrated tubercle bacilli on to the L. J. Culture and incubating at 37°C for 6 to 8 weeks. The growth of the bacilli was observed weekly. The *M. tuberculosis* strain H37Rv was used as a control for the tests. The tests were performed as per the methods given in the NTI Manual (1998).

Confirmation of *M. tuberculosis* by Biochemical and Molecular Biology Tests

The mycobacterium isolates which grew successfully on L. J. Culture Slants were further confirmed for their belonging to *M. tuberculosis*. The biochemical confirmation of the Culture Positive tubercle isolates was carried by Nitrate Reduction Test (Virtanen, 1960) and Niacin Production Test (Runyon *et al.*, 1974) while the Molecular confirmation was performed by Polymerization Chain Reaction (Eisenach *et al.*, 1991).

Nitrocefin Test For -Lactamase Production

The presence of -Lactamases in the mycobacterial clinical isolates was detected by reacting the cell suspension with nitrocefin that changes from yellow to red on hydrolysis. A 0.5 mM nitrocefin solution was prepared in dimethylsulphoxide (DMSO) and phosphate buffer. An inoculating loopful of colonies were spread directly from a LJ Slant and suspended in 20 µL volumes of 0.1 M phosphate buffer pH 7.0, to produce a dense suspension on a glass slide to which 20 µL of nitrocefin solution was added. The reaction was allowed to proceed for a maximum of 15 minutes and a colour change from yellow to red indicated β-lactamase production.

Analysis of Anti-mycobacterail Potential of Extended Spectrum -Lactam Antibiotic Against *M. tuberculosis*

The anti-mycobacterial potential of Extended Spectrum β-Lactam antibiotics against *M. tuberculosis was* confirmed by disc diffusion assay using discs impregnated with Extended Spectrum Penecillins and Broad Spectrum Cephalosporines (Wallace *et al.*, 1979). The antibiotics used included carbenicillin (100µg), mezocillin (75µg), piperacillin (100µg), ticracillin (50µg), cefotaxime (25µg), and ceftriaxone (30µg) and were purchased from Glexo and B.D. The susceptibility assay was performed by using antibiotics alone and also in combination with β-lactamase inhibitor clavulaniate in the ratio of 2:1.

The susceptibility of *M. tuberculosis* isolates to these antibiotics alone and the change in susceptibility on use of the antibiotics with β-lactamase inhibitors were determined by measuring the zone of inhibition appeared. H37Rv strain of *M. tuberculosis* was used as a control for the assay.

For performing the disc-diffusion assay, the *M. tuberculosis clinical isolates confirmed by different biochemical and molecular biology tests were used. Initially,* 10 ml of Middlebrook 7H9 broth medium was inoculated with a loop full of primary *M. tuberculosis* culture grown on L. J. slants. *The M. tuberculosis* cultures were grown up to late exponential phase in the

Middlebrook 7H9 broth. The cells were pelleted and washed in fresh medium and re-suspended in 10 ml fresh medium. Then, 150 l of washed culture was spread on Middlebrook 7H10 Agar medium and the antibiotic discs were placed. Plates were incubated for 2 weeks in a BOD incubator at 37^0C and zones of inhibition were measured to the nearest 5 mm.

RESULT

Isolation and Confirmation of *M. tuberculosis*

From 250 microscopically clinical sputum samples, only 241 sputum samples were found positive for *M. tuberculosis* in the ZN-staining and were selected for further study. Further, on the Primary L.J. culture (Fig-1) only 234 samples showed a visible growth. Then on the basis of Niacin Production and Nitrate reduction tests and Polymerase Chain Reaction (Fig-2), finally 233 *M. tuberculosis* clinical samples were used for antibiotic susceptibility assay.

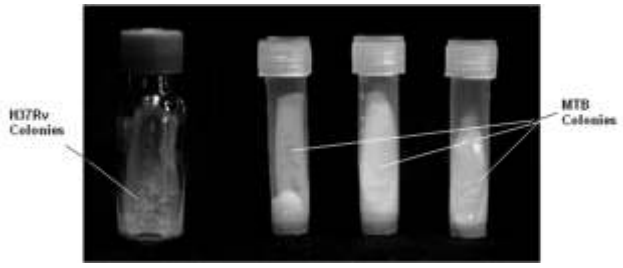

Fig- 1: L J Culture of *M. tuberculosis* Clinical Isolates and Control H37Rv

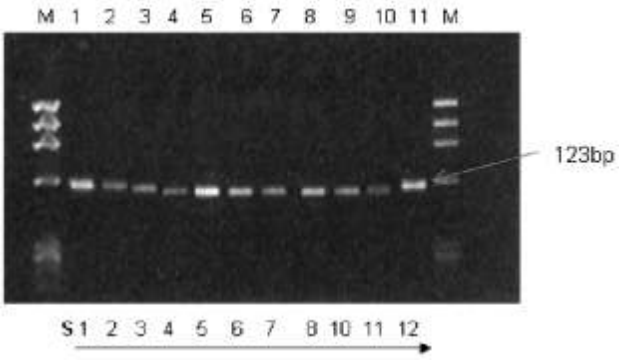

Fig.- 2: Polymerase Chain Reaction (PCR) Based Identification of *M. tuberculosis* in Clinical Samples (Sputum)

M= Molecular Marker for DNA Size Determination

S= Samples Collected From Saharanpur

Nitrocefin Test For -Lactamase Production

The results of nitrocefin test showed that all the 233 *M. tuberculosis* clinical isolates as well as the control H37Rv were able to produce β-Lactamase enzymes. (Fig.-3)

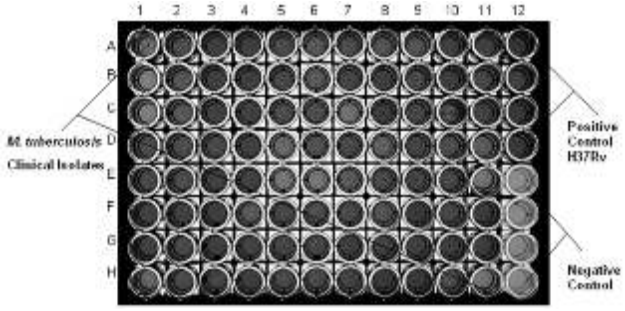

Fig.- 3: Nitrocefin Test for Screening of b-lactamases in M. tuberculosis (Column 1-11 and 12 (A-D), Control H37Rv (Column 12 A-D) Negative Control (Column 12 E-H)

Antibiotic Susceptibility Assay For Anti-mycobacterial Potential of Extended Spectrum -Lactamases Against *M. tuberculosis*:

The results of the Antibiotic Susceptibility Assay, showed that no zone of inhibition appeared for any of the 233 numbers of *M. tuberculosis* clinical isolates when antibiotics Carbenicillin, Mezocillin, Pipracillin, Ticracillin were used alone. However, a zone of inhibition ranging from 0-7 mm was observed for cefotaxime alone, while for ceftriaxon a zone of inhibition ranging from 13 to 17mm was observed. Almost similar results were observed for control H37Rv.

On using clavulanic acid (CA) as an inhibitor of β-lactamases along with the antibiotics, several time increase in the zone of inhibition has been observed. The zone of inhibition has been found 18-24 mm for carbenicillin + CA, 12-15 mm for Mezocillin + CA, 10-13 mm for Piperacillin + CA, 11-14 mm for Ticracillin + CA, and 12-16 mm for Cefotaxime + CA

However, no significant increase in the zone of inhibition has been observed for ceftriaxone + C.A. The similar results were also found for the control H37Rv. (Figure-4) (Table-1)

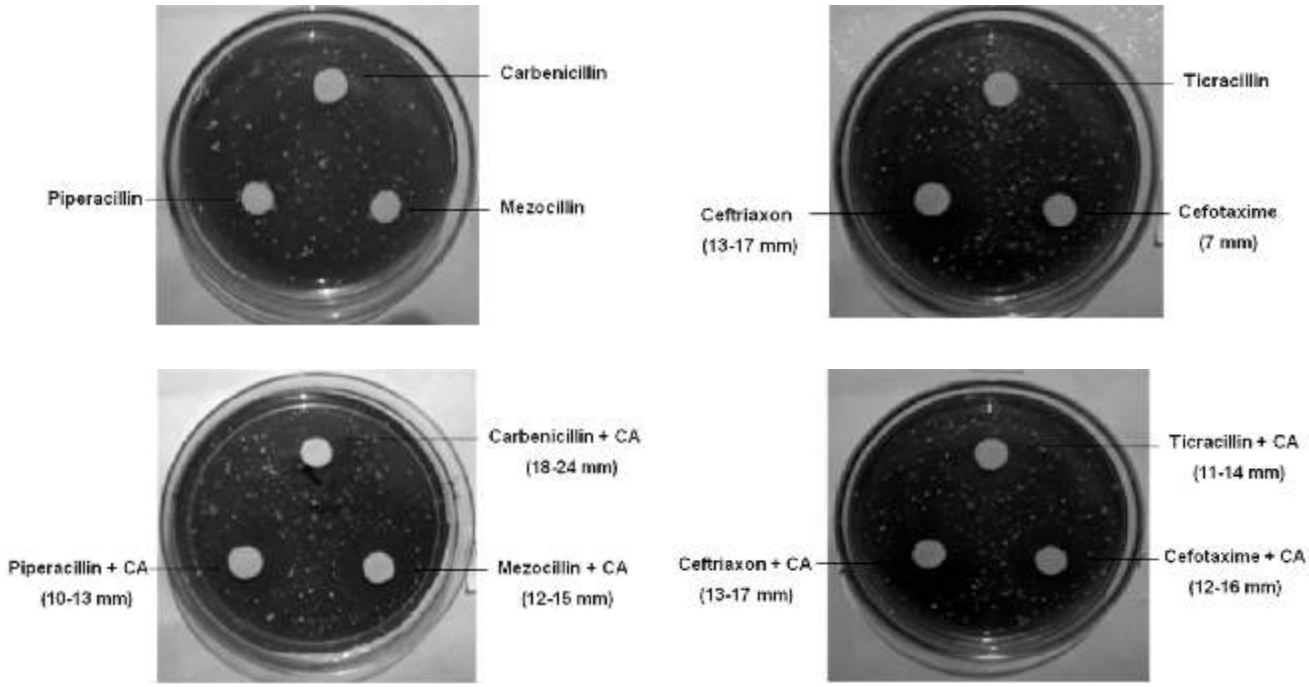

Fig- 4: Anti-Tuberculosis Potential of Extended Spectrum and Third Generation Broad Spectrum β-lactam Antibiotics
Extended Spectrum β-lactam Antibiotics – Carbenicillin, Mezocillin, Piperacillin and Ticracillin
Third Generation Broad Spectrum β-lactam Antibiotics – Cefotaxime, Ceftriaxon
CA = Clavulanic Acid

Table- 1: Result of Antibiotic Susceptibility Assay for Clinical Isolates of M. tuberculosis and Control H37Rv

ESBL Antibiotics (Amount Per Disc)	Zone of Inhibition (mm) For *M. tuberculosis*	
	Clinical Isolates N = 233	H37Rv (Control Strain 4 Sets)
—	00	00
Carbenicillin (100?g) + C.A. (50?g)	18-24	25
Mezocillin (75 µg)	00	00
Mezocillin (75 µg) + C.A. (37.5 µg)	12-15	15
Piperacillin (100µg)	00	00
Piperacillin (100µg) + C.A. (50µg)	10-13	13
Ticracillin (50 µg)	00	00
Ticracillin (50 µg) + C.A. (25 µg)	11-14	15
Cefotaxime (25 µg)	0-7	07
Cefotaxime (25 µg) + C.A. (12.5 µg)	12-16	15
Ceftriaxone (30 µg)	13-17	15
Ceftriaxone (30 µg) + C.A. (15µg)	20-24	17

ESBL- Extended Spectrum β-lactam C.A. – Clavulanic Acid

DISCUSSION:

The results of Antibiotic Susceptibility Assay showed that there was no inhibition of growth of *M. tuberculosis* when Extended Spectrum β-Lactam antibiotics Carbenicillin, Mezocillin, Pipracillin, and Ticracillin were used alone. However, Broad Spectrum βeta-Lactam antibiotics cefotaxime and ceftriaxon inhibited the growth of *M. tuberculosis*. When these antibiotics were used in combination of inhibitor of β–lactamases like clavulanic acid they significantly inhibited the growth of *M. tuberculosis* as shown by several times increase in the zone of inhibition. The inactivation of extended spectrum β-lactam antibiotics was due to the production of extended spectrum β–lactamases enzymes in the clinical isolates of *M. tuberculosis. The ESBL enzymes* can be inactivated by clavulanic acid. These extended spectrum β–lactamases enzymes do not affect the activity of most of the Broad Spectrum β-Lactam antibiotics which is apparent from the results for cefotaxime and ceftriaxon where no significant increase in the zone of inhibition could appear when these antibiotics were used in combination with clavulanic acid. These results support the of finding of earlier workers like Chambers *et al.* (1995) who observed that penicillins and other beta-lactam antibiotics like amoxicillin and imipenem inhibit the growth of *M. tuberculosis* when used beta-lactamase inhibitor like clavulanate and sulbactum, Dinçer *et al.* (2004) reported antituberculosis activity of β-lactam antibiotic cefazolin-clavulanic acid, while Anthony *et. al.* (2005) observed the susceptibility of *M. tuberculosis* and *M. smegmatis* with various to β-lactam antibiotics. Hugonnet *et al.* (2009) for lactam antibiotic meropenem combined with clavulanic acid against extensively drug resistant *M. tuberculosis.*

Since, the present study proves that the Extended Spectrum β-Lactam antibiotics have significant anti-mycobacterial potential, these antibiotics can be used as a regimen for the treatment of *M. tuberculosis* infections specifically in the events of rapidly increasing HIV/TB coinfections where the patients are not in the very good physiological condition to tolerate the strong antibiotics or chemotherapy for TB and also in the case of MDR or XDR tuberculosis.

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Management of a Severely Resorbed Mandibular Ridge with Internally Weighted Denture Base Using a Cast Metal Insert and a Neutral Zone Recording

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Abstract : The present investigation describes a method for fabrication of cast metal insert which is comfortable and has an edge over conventional acrylic resin denture and other surgical implantation in patients for severe resorption of mandibular alveolar ridge. In this method, fabrication of internally weighted mandibular complete denture using a cast metal insert in a processed denture base and neutral zone recording for teeth arrangement has been described. The method has shown improved retention and stability of the prosthesis along with the level of satisfaction achieved. Sequential clinical and laboratory procedures to incorporate a solid metal frame at a predetermined, controlled position within the prosthesis are presented. This procedure describes the design, weight and position of the metal base to be customized for an optimal esthetic and functional outcome, and allows for conventional reline procedures.

Key words: Weighted denture, Acrylic resin templates, Physiologic impression, Retention, Stability, Relining, Esthetics.

INTRODUCTION

Severe resorption of the mandibular alveolar ridge contributes to instability and discomfort of the conventional acrylic resin denture. Alveolar ridge resorption may be corrected, in part, by various surgical implantation and vestibuloplasty techniques. When these techniques are not feasible, the need to construct a mandibular denture that is strong, stable, and functional can be met by a metal based denture.

Weighted metal bases have been used to manage the unfavorable mandibular residual ridge and to reinforce the mandibular denture base (Faber, 1957; Strahl *et al.* 1984; Belfiglio,1987). Grunewald (1964) introduced gold as the metal of choice for the resorbed mandibular residual ridge. He compared the weight of the average gold base with the weight of the teeth and bone lost through extraction and extensive resorption and suggested a gold base of approximately 16 dwt (25 g) for the average-sized mandible is a better choice. DeFurio *et al.* (1970) reported that chrome-cobalt was the most retentive base material for maxillary complete dentures. Significant disadvantages of metal base dentures are that they often irritate the underlying alveolar ridge and are difficult to reline and adjust. Massad (1987) introduced a metal base denture with a resilient liner to manage the severely resorbed mandibular residual ridge. Garfield (1984) suggested an acid-etch technique to reline metal-based prostheses. Other techniques have also been proposed for fabricating and positioning the internal metal bases during denture processing to overcome the disadvantage of the metal base. Wormley *et al.* (1974) described a technique to fabricate internally weighted mandibular dentures using either chrome-cobalt alloy or gold.

Hurtado (1988) and Kim *et al.* (2009) described some techniques to overcome the disadvantage of the metal base for fabricating and positioning the internal metal bases during denture processing. They have also advocated that when ridge resorption is excessive, ridge crest is unreliable guide for teeth placement. The greater the ridge loss, the smaller the denture base area and the less influence the impression surface area will have on the stability and retention of the denture. As the area of the impression surface decreases and the polished surface area increases, tooth position and contour of the polished surface become more critical. Therefore, these surfaces should be so contoured that the horizontally directed forces applied by the peri-denture muscles should act to seat the denture. The weight, design, and inherent strength of such a denture meets the patient's special needs has suggested by Pasam *et al.* (2006). This should thus be recorded by using neutral-zone philosophy (Fish, 1948; Beresin *et al.*, 2006).

This paper describes a method for fabricating an internally weighted mandibular complete denture using a cast metal insert in a processed denture base and neutral zone recording for teeth arrangement. The method is then evaluated for its efficacy in improving the retention and stability of mandibular complete denture and patient satisfaction.

Materials and Methods

This method was applied and studied in five patients, aged 50-70 years who were quite unsatisfied with their mandibular complete denture and presented with severe mandibular ridge resorption and retracted

Figure 1. Self cure acrylic resin templates

tongue position.

The detailed procedure is described as under:

1. A full-coverage mucostatic mandibular impression was made by using stock impression trays and irreversible hydrocolloid (Tropicalgin, Zhermack, Italy) impression material.
2. The impression was poured with type 3 dental stone (Denstone, Zhermack, Italy).
3. Self cure acrylic resin template (Acralyn, Asian Acrylates, Mumbai, India) with increased thickness was delivered to the patient over a period of three weeks. (Figs.1&2) (Acrylic resin templates with increasing thickness were given to patients to get adapted to increased weight of the final prosthesis).

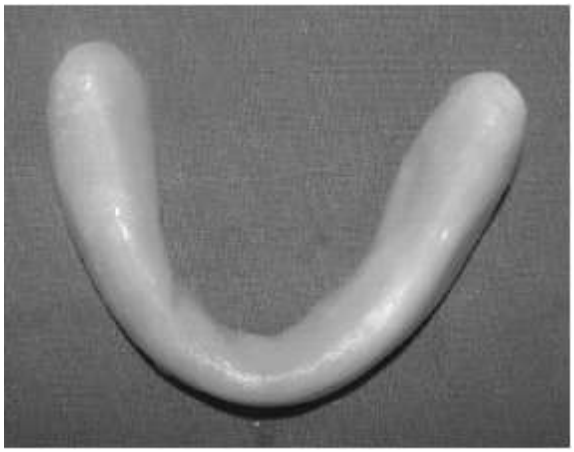

Figure 2. **Self Cure acrylic resin template**

4. Physiologic impression was made with soft liner using acrylic resin template as custom tray(Fig.3).

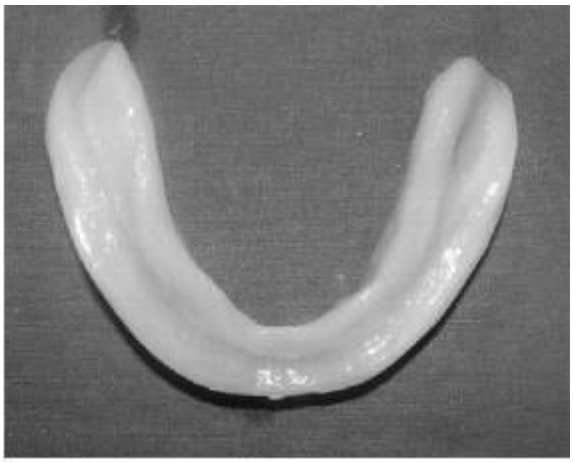

Figure 3. **Physiologic impression made with soft liner**

5. The impression was poured with type 3 dental stone (Denstone, Zhermack, Italy).
6. Double thickness of base plate wax (Rolex, Ashoo Sons, New Delhi, India) was adapted over the entire edentulous ridge of the cast to create a processed denture base, as described by Graser (1978). Upper single thickness of baseplate wax was cut to incorporate the shape of a subsequent wax insert for the metal base. (Fig.4)
7. The cast with adapted baseplate wax was duplicated with VPS impression material (Wirosil; BEGO USA, Lincoln, RI) and poured in investment material (Wiroplus S; BEGO,USA).
8. A custom wax insert (Rolex, Ashoo Sons, New Delhi, India) was fabricated on the refractory cast to provide optimal base fit and improve the esthetic outcome. Beads were added to increase retention of acrylic resin as described by Massad (1987) (Fig.5).

Figure 4. **Baseplate wax adapted on master cast with custom metal insert shape cut out in second layer of baseplate wax**

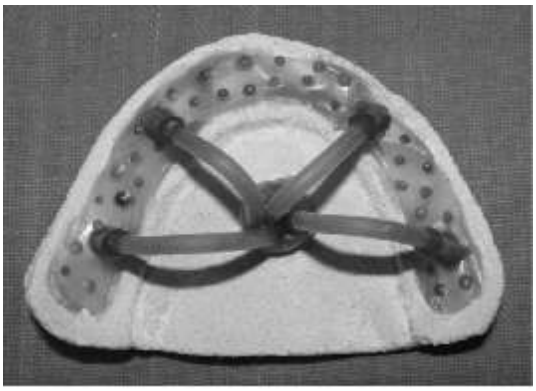

Figure 5. **Wax pattern of custom metal insert on refractory cast with sprues**

9. The wax pattern was weighed to calculate a suitable metal weight, according to Grunewald's recommendation. The pattern was adjusted after considering the patient's gender, age, and residual bone mass and was casted in base metal (Wironium BEGO USA).

10. Finger-like extensions were made all around the periphery of cast metal insert and was then adapted over the first single thickness of baseplate wax (adapted on the master cast).

11. The wax trial denture base with adapted cast metal insert was processed with high impact heat-polymerized acrylic resin (Trevalon-HI, Dentsply, Germany).Acrylic studs are made over metal insert to maintain its position during processing (Fig.6)

12. The occlusal vertical dimension of the patient was determined using permanent denture base with wax occlusal rims.

Figure 6. **Custom metal insert adapted on wax trial denture base with acrylic studs**

13. Casts were mounted with a facebow and centric relation record (Occlufast, Zhermack, Italy) using the split-cast method described by Lauritzen *et al.*(1964).

14. Mandibular wax rim was then modified to record neutral zone with compound rims to further enhance the stability of the prosthesis. Putty index of neutral zone was made and denture teeth were arranged (Acryrock, Ruthenium, Italy). Trial dentures were then evaluated clinically (Fig.7&8).

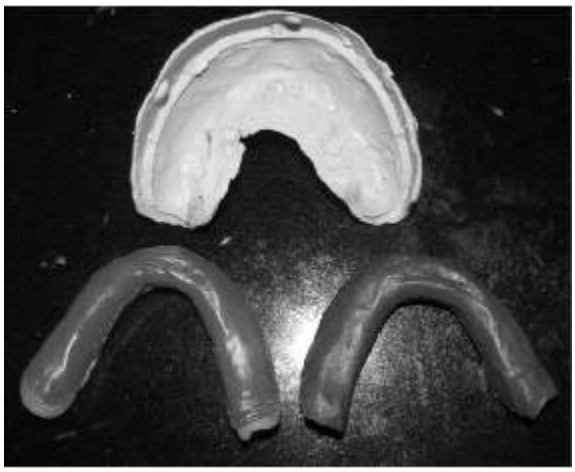

Figure 7. **Neutral Zone recorded with compound rims and putty index was made to replace rim with wax**

15. After the trial placement was assessed to be satisfactory, trial dentures were processed with high impact heat-polymerized acrylic resin (Trevalon-HI, Dentsply, Germany). The prosthesis was retrieved and polished for the denture placement (Fig.9).

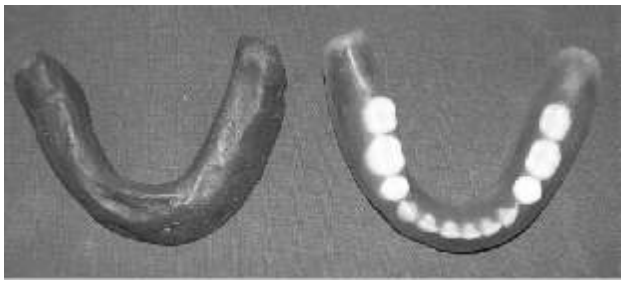

Figure 8. Trial denture with teeth arranged according to neutral zone

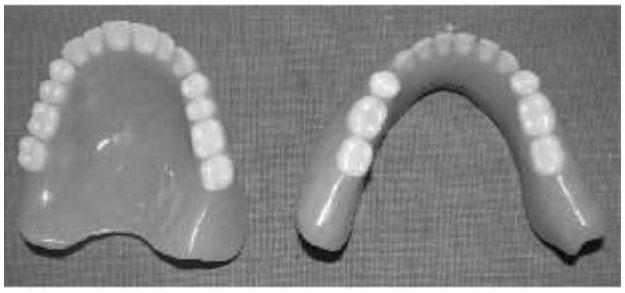

Figure 9. High impact heat-polymerized acrylic resin Processed dentures

Results and Discussion:

Weighted metal bases have been used to manage the unfavorable mandibular residual ridge and to reinforce the mandibular denture base. A simplified fabrication technique for an internally weighted denture base has been described which is modified to the procedure described by Pasam *et al.* (2006) and Beresin *et al.* (2006) . In this method, since permanent denture base with cast metal insert has been made before recording jaw relation, both the jaw relation and neutral zone are recorded more accurately on stable denture bases. Also the cast metal insert has been customized making teeth arrangement and final wax up to be performed easily over stable permanent denture base and thus giving excellent esthetics without the need of masking the colour of base metal as compared to the techniques described by Jameson (2000) and Kim *et al.* (2009). For patients described in this article, the average internally weighted metal base weighed 18.5 grams, or 12 dwt (2.2 grams of base plate wax x base metal density of 8.4 grams/cm3), and the average weight of the mandibular prosthesis has been found to be 32.4 grams (20.8 dwt) which is in accordance with recommended weight (Grunewald, 1964). A significant advantage of this technique is that the design, weight and position of the metal base is customized at the clinician's request for an optimal esthetic and functional outcome, and allows for conventional reline procedures. The predictable laboratory technique described may benefit the patient with a minimal residual ridge when implant therapy or preprosthetic surgery is not an option. The patients evaluated in this study showed improved functional outcome and greater satisfaction with their mandibular complete denture due to increased stability and retention of the prosthesis.

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Tannin Yielding plants of Central Narmada Region in India

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Abstract

The present study mainly focuses on some Tannin yielding plants from central Narmada region of India. About 220 plant species were screened for the presence of tannin, out of these 83 species belonging to 64 genera and 30 families showed positive results for tannins. The botanical names, family, vernacular name and parts from which tannin is obtained are described in this paper.

Key words: Tannin yielding Plants, Central Narmada Region

Introduction

Central Narmada valley is situated between Vidhya and Satpuda hills of Central India. This region lies between $21^0$54 to $22^0$59E longitudes and $76^0$46 to $78^0$42 N latitude The average height of this region is ±331 meters form sea level. This region has rich biodiversity. The region is the centre for collection of tanning material since long. The word tannin is very old and reflects a traditional technology. "Tanning" (water proofing and preserving) is the word used to describe the process of transforming animal hides into leather by using plant extracts from different sources. Tannin have been used in tanning animal skins to make leather; it transforms certain proteins of animal tissue into compounds that resist decomposition. It is also used in manufacturing inks, as a mordant in dyeing, and in medicine as an astringent and for treatment of burns. Tannins are generally obtained from barks, wood, fruits, pods, and leaves some plants like 'Babool'(*Acacia nilotica)*, 'Khair' (*Acacia catechu)*, 'Arjun' (*Terminalia arjuna*.), 'Harra' (*Terminalia chebula*) 'Eucalyptus' (*Eucalyptus* sps.), and 'Ber' *(Zizyphus* sp.). Tannins are found in the vacuoles or on the surface of plants along with wax. They protect the plants from being eaten away by the animals and support the plant metabolism. Review of available literature shows that tannin yielding plants are not properly studied with reference to central India. (Jain,1991, Upadhyay, 2005, Pandey and Kori, 2009.) Present work is undertaken to study the tannin yeilding plants of Central Narmada Region.

Materials and method

The present study is the outcome of exhaustive field survey under taken for the period of two years from 2008 to 2010. About 220 plants were collected from different places of central Narmada region in central India. The plant material was collect from Pilikarar, Pandadoh, Khandawar, Joshipur, Barkheda , Basapur, Goradia, Mahukala, Patalkhoh, Shahpura, in Sehore district and Malakhedi, Raipur, Jasalpur Bandhrawan, Randhar, Hashalpur , Baduba in Hoshangad district in various seasons. Some important information was gathered from the local and tribal people. Descriptions of species and identification were done with the help of various flora and available literature, especially Cooke (1975), Oommachan (1977), Kaushik (1983), Maheshwari (1963), Mukherjee (1984), Randhva (1983), Deshpande and Singh (1986) and Verma *et al.* (1993) and Dictionary of Indian folk medicine and ethno-botany (Jain, 1991) The species were identified and the voucher specimens were deposited in the Herbarium of Botany Department of Government Narmada Post-Graduate College, Hoshangabad, (MP).

The plant material like leaves, barks, fruits and seeds were collected and dried under the shade. This plant material was used for phyto-chemical studies. Extracts of the plant materials were prepared by boiling them in distilled water over gas burners. The extracts were cooled in water bath and tested for the presence of tannins by using 5% $FeCl_3$ and 5% Potassium dichromate.

Observations Table -1

S. No.	Tannin Yielding Plant	Family	Parts having tannin	Vernacular Names
1	*Abutilon indica (L.) Sweet.*	Malvaceae	Flower	Kanghi , Mahabala
2	*Abutilon graveolens W&A*	Malvaceae	Leaves	Kanghi , Mahabala
3	*Acacia catechu (L.) Wild ,*	Mimosaceae	Bark, wood	Cattha
4	*Acacia leucophloea L.*	Mimosaceae	Bark	Rinja
5	*Acacia nilotica (L.) Del*	Mimosaceae	Bark, Pods,	Babool
6	*Adhatoda vasica (Linn.) Nees*	Acanthaceae	Bark and flower	Adusa
7	*Aegle marmelos Correa*	Rutaceae	Bark	Bel
8	*Albizia lebbeck (L.) Bth*	Mimosaceae	Bark	Siris
9	*Albizia procera (Roxb.) Benth*	Mimosaceae	Bark	Safed Siris
10	*Alangium salvifolium (L.f.)Wangerin*	Alangiaceae	Flower, Bark, Leaves	Aankol
11	*Annona reticulata L.*	Annonaceae	Bark	Ramphal
12	*Annona squamosa L.*	Annonaceae	Bark	Sitaphal
13	*Artocarpus heterophyllus Lamk.*	Moraceae	Bark	Kathal
14	*Bauhinia variegata L*	Caesalpinaceae	Flower	Kachnar
15	*Bauhinia recemosa Lam*	Caesalpinaceae	Bark	Sirhatta
16	*Buchanania lanzan Roxb.*	Anacardiaceae	Bark	Achar
17	*Bombax ceiba L.*	Bombacaceae	Bark	Semul
18	*Boerhavia difusa L.*	Nyctaginaceae	Whole plant	Punarnava
19	*Butea monosperma (Lamk.) Taub.*	Paplionaceae	Bark, Flower	Palash
20	*Butea superba Roxb.*	Paplionaceae	Bark, Flower	Bel Palash
21	*Cassia siamea L.*	Caesalpinaceae	Flower, Bark, Leaves	Vilaiti amaltas
22	*Cassia tora (Linn.)Linn*	Caesalpinaceae	Seed, Leaves, Barks	Chakodar , Puwar
23	*Cassia fistula L.*	Caesalpinaceae	Bark	Amaltash
24	*Callistemon citrinus L.*	Myrtaceae	Bark	Bottal-Brush
25	*Cordia dichotoma Forst.f.*	Cordiaceae	Bark	Lasora , Gondi
26	*Cordia meclleidii Hook.f.& Thoms*	Cordiaceae	Bark	Dhahiman
27	*Cymbopogon citratus Stapf.*	Poaceae	Leaves	Lemon Grass
28	*Dalbergia sissoo Roxb*	Fabaceae	Bark	Sesam

29	Dalbergia latifolia Roxb	Fabaceae	Bark	Kala Sesam
30	Depteracanthus suffruticosus (Roxb.)	Acanthaceae	Flower	….
31	Diospyros malenoxylon Roxb.	Ebenaceae	Bark	Tendu
32	Datura alba Mill.	Solanaceae	Leaves	Dhatura
33	Datura metel L.	Solanaceae	Flower	Kala Dhatura
34	Eclipta alba (L.)Hassk.	Asteraceae	Whole plant	Bhringraj
35	Emblica officinalis Gaerthn.	Euphorbiaceae	Bark	Amala
36	Eugenia jambolana Lam	Myrtaceae	Bark	Jamun
37	Euphorbia hirta L	Euphorbiaceae	leaves , Stem, Flower	Dudhi
38	Evolvulus alsinoides (L.) L.	Convolvulaceae	Leaves	Sankpuspi
39	Ficus glumerota Wall. Ex Roxb	Moraceae	Bark	Gooler
40	Ficus cupuleta Roxb	Moraceae	Bark , Leaves	Bargad
41	Ficus ruffa L	Moraceae	Bark	Gangli Gooler
42	Ficus retusa L.	Moraceae	Bark	Fafer
43	Hibiscus rosa-sinensis L.	Malvaceae	Flower	Gudhal , Jason
44	Ipomoea aquatica Forsk	Convolvulaceae	Flower	Beshram
45	Ipomoea Carnea Jacq. Ssp. Fistulosa (Mort. Ex. Choisy) D. Austin	Convolvulaceae	Flower	Beshram
46	Jatropha basifolia L	Euphorbiaceae	Leaves	Rattanjot
47	Jatropha curcas L	Euphorbiaceae	Leaves	Rattanjot
48	Launea procumbens (Roxb) Ram. & Raj.	Asteraceae	Leaves	Banghobhi
49	Lawsonia inermis L.	Lythraceae	Leaves , Seed, Barks	Heena , Mehendi
50	Madhuca indica J.F.Gmel	Sapotaceae	Bark	Mahua
51	Manilkara hexandra (Roxb.) Dub	Sapotaceae	Bark	Rori
52	Mangifera indica L	Anacardiaceae	Bark , Leaf	Aam
53	Mimosa pudica L.	Mimosaceae	Bark	Lajbanti , Chhuimui
54	Parthenium hysterophorus L.	Asteraceae	Whole plant	Gajarghass
55	Phyllanthus faternus Webst.	Euphorbiaceae	Bark	Bhui - Amla
56	Psidium guajava L.	Myrtaceae	Bark	Jam
57	Pterocarpus marsupium Roxb	Fabaceae	Bark	Beejsal

58	Phoenix sylvestris Roxb.	Arecaceae	Leaves	Chhinda
59	Poinciana regia L.	Caesalpinaceae	Flower	Gulmohar
60	Punica granatum L.	Punicaceae	Fruit rind	Anar
61	Randia dumetorum (Retz.) Poir.	Rubiaceae	Bark	Phender
62	Ricinus communis L.	Euphorbiaceae	Leaves	Arandi
63	Ruellia tuburosa L	Acanthaceae	Flower	……
64	Senecio chrysanthemides DC	Asteraceae	Leaves	……
65	Solanum indicum L.	Solanaceae	Fruit	Bhatt - Katai
66	Solanum nigrum L.	Solanaceae	Flower and Leaf	Makoi
67	Solanum xanthocarpum Schard & Wendl	Solanaceae	Leaves	Neeli - Kateri
68	Sonchus asper (L.) Hill.	Asteraceae	Leaves	Bangobhi
69	Sphaeranthus indicus L.	Asteraceae	Inflorescence	Gorak-Mundi
70	Murraya koenigii (Linn.) Spreng.	Myrtaceae	Bark , Leaves	Mithi-Neem
71	Tamirandus indica L.	Ceasalpinaceae	Leaves, Bark	Imali
72	Tectona grandis L.	Verbenaceae	Leaves	Sagoan
73	Terminalia arjuna (Roxb. Ex DC.) wt & Ann.	Combrataceae	Bark	Arjun,Kahu
74	Terminalia bellerica (Gairth.) Roxb.	Combrataceae	Fruit	Bahera
75	Terminalia chebula Retz.	Combrataceae	Fruit	Harra
76	Terminalia tomantosa (DC.) wt. & Arn.	Combrataceae	Bark	Saja
77	Tridex procumbens L.	Asteraceae	Leaves , stem	…
78	Ventilago denticulata Willd.	Rhamnaceae	Bark	Kevti
79	Vitex negundo L.	Verbenaceae	Leaves	Nirgundi
80	Wrightia tinctoria Br.	Apocynaceae	Bark	Dudhi
81	Wrightia tomantosa. Raemi.& Schult.	Apocynaceae	Bark	Dudhi
82	Zizyphus mauritiana Lamk.	Rhamnaceae	Bark	Ber
83	Zizyphus nummularia (Burm.) wt. & Arn.	Rhamnaceae	Bark	Jhar-Beri

Result and discussion

Central Narmada valley is one of the floristically richest regions in Central India. In present work 83 species of Angiosperm belonging to 64 genera and 30 families were found to contain tannins. Among the plant studied, tannins are more common in Dicotyledons than in Monocotyledons. Only one species (*Cymbopogon sps*) of monocot tested positive for tannins while 82 species belong to 29 families of dicotyledon has tannins. Asteraceae , Caesalpinaceae is found to be dominant with 8 species followed by Asteraceae with 7 speceis , Euphorbiaceae with 6 species, Mimosaceae with 5 species ,Moraceae, Solanaceae with 5 species each, Combrataceae and Myrtaceae with 4 species, Acanthaceae, Convolvulaceae ,Fabaceae Malvaceae Rhamnaceae with 3 species each and Anacardiaceae, Annonaceae, Apocynaceae, Fabaceae, Sapotaceae and Verbenaceae with 2 species each. The remaining 10 families have one species of tannin yielding plant.

Tannins are used in India's native Ayurvedic medicine for centauries, primarily as a cardiac tonic(Anon,1952). Tannins of *Terminalia arjuna* were studied by various workers (Kumar and Prabhakar,1987) Clinical trials indicates that it can be beneficial in the treatment of coronary artery disease, heart failure, and possibly hypercholesterolemia(Alpana *et al.*, 1997) and pharmacological studies show that Antidyslipidemic and antioxidant activities of this plant is mainly due to the tannins present in its bark.(Chandra *et al.*, 2004). Tannin content alters during the development of the plant and also as a response to the environmental changes. (Salminen *et al.,* 2001*)* These variations influence directly the quality of the plant for medicinal use. (Santos *et al.*,2002). Tannin transforms certain proteins of animal tissue into compounds that resist decomposition.

Commercialization of tannin can be successful in the central Narmada region with systematic and scientific approach for identification of resources, extraction, purification, chemical structure elucidation and promotion of use of tannin, there by enhancing the economy of the local people. As a whole, systematic approaches with scientific attitude would help in conserving the economically important plant resources, in addition to the rich indigenous knowledge base available in this region.

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Toxicity of *Dryopteris filix*-mas powder against the ontogeny of rice-moth, *Corcyra cephalonica* (Staint.) (Lepidoptera: pyralidae)

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Abstract.

Larvicidal and pupicidal effects of *Dryopteris filix*-mas (root and rhizome) powder was made on the third instar larvae of *Corcyra cephalonica* (Staint.). The observations revealed that 4% dose of *Dryopteris filix-mas* caused 100% larval mortality indicating absolute toxicity to the pest**.**

Keywords. *Dryopteris filix*-mas; *Corcyra cephalonica*; Ontogeny; Toxicity;

Introduction

Stored insect pests are a problem throughout the world, because they reduce the quantity and quality of grain. Out of these, the rice-moth, *Corcyra cephalonica* (Staint.) is a notorious pest of stored cereals and cereal commodities in India as well as in other tropical and subtropical regions of the world. This moth was first identified and reported by Stainton (1866), who named it *Melissoblaptes cephalonica*. Later, Ragonot (1885) gave its generic name as *Corcyra*. The only recognized species of this genus is *cephalonica*. Ayyar (1919) made the first record of *Corcyra cephalonica*. This moth is believed to be of eastern origin but has become a cosmopolitan species. Its larval stages cause appreciable loss to rice, sorghum, maize, currants, grams, cocoa beans, peanuts, cottonseeds, linseeds, raisins, chocolates, army biscuits, nutmeg and milled products. (Chittenden, 1919; Ayyar, 1919; Munro and Thompson, 1929; Richards and Herford, 1930; Noyes, 1930; Herford, 1933; Atwal, 1976 and Piltz, 1977).

Sufficient knowledge exists on the nutritional and reproductive physiology of this lepidopterous pest (Krishna and Narain, 1976; Bhatt and Krishna, 1980, 1982, 1984 a, b,c, 1986; Srivastava and Krishna, 1976, 1978 and Mishra and Krishna, 1980). In addition, influence of insecticidal agents like organochlorines, organophosphates and a few synthetic pyrethroids have also been reported against the ontogeny as well as larval biochemistry of this lepidopterous pest (Tiwari and Bhatt, 1987; 1994 a,b,c; 1999 a, b, c; 2000; Tiwari and Tripathi, 2006). Although, the use of organophosphorus and organochlorine insecticides pose problems such as poisoning in man and other animals pest (Wiriyachitra and Philongene, 1993), resistence to pesticides (Chand and Pratap, 1977), the risk of user's contamination, injurious to non-target organisms and even cause pollution to our environment and hence disturbing the ecosystem. Thus, there is an urgent need to develop safe alternatives to conventional insecticides for the protection of grain and grain products against insect infestations.

Plant products, which show diverse biological activities, may be useful for this purpose. Higher plants are a rich source of novel natural substances that can be used to develop environment safe methods for insect control (Jbilou *et al*., 2006). Plant materials with insecticidal properties have been used traditionally for generations throughout the world, for a number of reasons (Belmain *et al*., 2001; Golob *et al*., 1999; and Weaver and Subramanyam, 2000). Botanical insecticides compared to synthetic ones may be safer for the environment, are generally, less expensive, easily processed and used by farmers and small scale industries (Belmain *et al*., 2001). Since these plant materials with insecticidal properties are often active against a limited number of species, are often biodegradable to non toxic products, and are potentially suitable for use in integrated pest management, they could lead to the development of new classes of safer insect control agents (Kim *et al.*, 2003).

Use of vegetable oils against adult weevils of *Sitophilus oryzae* (L.) had been suggested by (Gupta *et al*., 2000). The foliage powder of *Calotropis gigantea*, *Ocimum sanctum*, *Iponoea fistulosa* and *Ipomoea carnea* had been found to be insecticidal against several stored product pest. (Ryan and Byrne, 1988; Weaver et

al., 1991; Chowdhary *et al*., 1997; Larshini *et al.,* 1997, Pari *et al.,* 1998; Solanki and Shanker, 2001 and Srinivasan *et al.,* 2003). Earlier findings reveal that the rhizome and young shoots (fiddleheads) of the male fern (*Dryopteris filix-mas*) have deworming properties that have long been recognized in Europe against tapeworms (*Taenia*). The ferns are effective in arresting embryonic development in insects. The extracts of pteridophytes have toxic effects on *Spodoptera littura* and *Helicoverpa armigera*. Filicin, isolated from the rhizome of *Dryopteris filix-mas,* has a potential insecticide (Mannan *et al*., 2008).

In the present study *D. filix*-mas has been selected as one of the safer substitutes to control the stored cereal pest rice-moth, *Corcyra cephalonica.* Hence, as an objective of such programme the present work has been designed and conducted to investigate the effect of *D. filix*-mas root and rhizome powder against the ontogeny of rice-moth, *Corcyra cephalonica*. Such knowledge may be regarded as one of the objective criteria permitting an assessment of effectiveness of botanical control measures against *Corcyra cephalonica* in particular and lepidopterous pests in general.

1. Materials and Methods

D. filix-mas plants were collected from adjacent areas of Gorakhpur and neighbour districts of U.P. Their rhizomes along with roots were separated from the plant body, properly washed with fresh tap water, cut into small pieces, cooked in boiling water for more than one hour to destroy the thiaminase, dried in sun light for six to seven days, pulverized in a mortar and pestle and then it was ground in an electric grinder. The powder so obtained was utilized in toxicological experiment.

From the laboratory maintained culture on ground jowar mixed with 5% (w/w) yeast powder, newly emerged males and females were transferred to oviposition glass chambers (35 mm diameter, 200 mm height). Eggs laid by the females were collected and then placed in glass chambers (consisting of 250 ml beakers) for hatching. Freshly hatched larvae of *Corcyra cephalonica* were allowed to feed on a normal dietary medium mixed with 5% yeast powder (w/w) kept inside 250 ml beakers for exactly 15days. On the 16^{th} day of larval hatching, 25 third instar larvae were transferred to each similar rearing chambers (250 ml beakers) containing 50 gms of dietary medium mixed and treated separately with 9 different doses i.e. 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, and 4 % of *D. filix-mas* root and rhizome powder, using 5 replication of each treatment and also a normal dietary medium as control. On the completion of developmental cycle, percent adult emergence and percent pupal death was observed and on that basis percent pupation and percent larval death was calculated. Experiments were replicated five times and the values have been expressed as the mean ± S.D. Straight line regression equation was applied between different concentrations of natural plant products and their corresponding percent larval death /percent pupation /percent pupal death and percent adult emergence to observe the significant correlation. Amount of insecticide consumed by larvae were calculated as µg/larva at each dose level of *D. filix*-mas root and rhizome powder. LD_{50} values (µg/larva), 95% confidence limits (lower and upper confidence limits) of Ld_{50}, slope values, g values and heterogeneity of *D. filix*-mas root and rhizome powder was calculated by Polo Plus, Probit and Logit Analysis, Version: 2.0, LeOra Software based on Probit analysis (Finney, 1959), Table 1.

It deserves mention that the dietary medium (roughly ground jowar, *Sorghum vulgare*) was mixed with 5% (w/w) yeast powder so in control dietary media there was no larval mortality resulting into 100% pupation and 100% adult emergence. Hence, there is no need to apply Abbott's formula.

Results

Results, presented in Table 2 and Fig. 1, revealed that a significant larval mortality was obtained with the increase of *Dryopteris filix-mas* root and rhizome powder dose. At 0.25% dose of *Dryopteris filix-mas* larval mortality was only 15 ± 1.80% while 100% larval mortality was recorded at 4.00% dose level of *Dryopteris filix-mas*. As the *Dryopteris filix-mas* dose increase a significant reduction in pupation and a significant enhancement in pupal death did occur. 85 ± 1.80% pupation was recorded at 0.25% dose which decreased to 4% % ± 2.82 at 3.5% dose of *Dryopteris filix-mas.* At the same time, 3.44 ± 2.23% pupal death was recorded at 1.00% dose level of *Dryopteris filix-mas*, which increased to 100% at 3.50% dose level of *Dryopteris filix-mas*. A significant reduction in adult emergence was recorded following exposure of increased dose levels of *Dryopteris filix-mas*. At 0.25% dose level of *Dryopteris filix-mas* 85 ±1.80% adult emergence was recorded that decreased to 2.0 ± 3.60% at 3.0% dose level of *Dryopteris filix-mas.*

Table 1. $LD_{10,}$ LD_{50} and LD_{90} values, Confidence Limits (LCL and UCL) of $LD_{50,}$ Slope Values, g Values and Heterogeneity of *Dryopteris filix*-mas root and rhizome powder to the 3rd- 5th instar larva of rice-moth, *C.cephalonica.*

Natural plant products	Effective doses (µg / larva)		Confidence limits LCL	UCL	Slope Values	g Values	Heterogeneity
D. filix-mas powder	LD_{10}	0.282	0.867	1.026	2.439 ± 0.118	0.009	0.99
	LD_{50}	0.946					
	LD_{90}	3.173					

Table 2**.** Toxicity of *Dryopteris filix-mas* root and rhizome powder against the ontogeny of rice-moth, *C. cephalonica.*

% *Dryopteris filix -mas* root and rhizome powder dose levels in food enriched with 5% (w/w) yeast powder	Percent* larval death	Percent* pupation	Percent* pupal death	Percent* adult emergence	Acute toxicity to the pest
Control	0	100	0	100	
0.25		85±1.80	0	85±1.80	Poorly toxic
0.50	26±3.60	74±3.60	0	74±3.60	Moderately toxic
1.00	42±2.23	58±2.23	3.44±2.23	56±4.00	Moderately severe
1.50	59±1.80	41±1.80	9.75±1.41	37±1.80	Moderately severe
2.00	70±2.23	30±2.23	30.0±2.23	21±1.80	Severely toxic
2.50	86±2.23	14±2.23	64.28±1.80	5.0±3.35	Severely toxic
3.00	93±1.80	7.0±1.80	71.42±3.35	2.0±3.60	Severely toxic
3.50	96±2.82	4.0±2.82	100	__	Extremely toxic
4.00	100	__	__	__	Extremely toxic

*Values have been expressed as the mean ± S.D. of five replicates.

Straight line regression equation was applied between different dose levels of *Dryopteris filix-mas* root and rhizome powder and their corresponding percent larval death/percent pupation/percent pupal death/percent adult emergence to observe the significant correlation:

Percent larval death $y = 12.855 + 25.120x$; $r = 0.97$ $P < 0.001$

Percent pupation $y = 89.7463 - 27.696x$; $r = -0.98$ $P < 0.001$

Percent pupal death $y = -14.804 + 28.921x$; $r = 0.94$ P insignificant

Percent adult emergence $y = 92.047 - 33.15x$; $r = -0.98$ $P < 0.001$

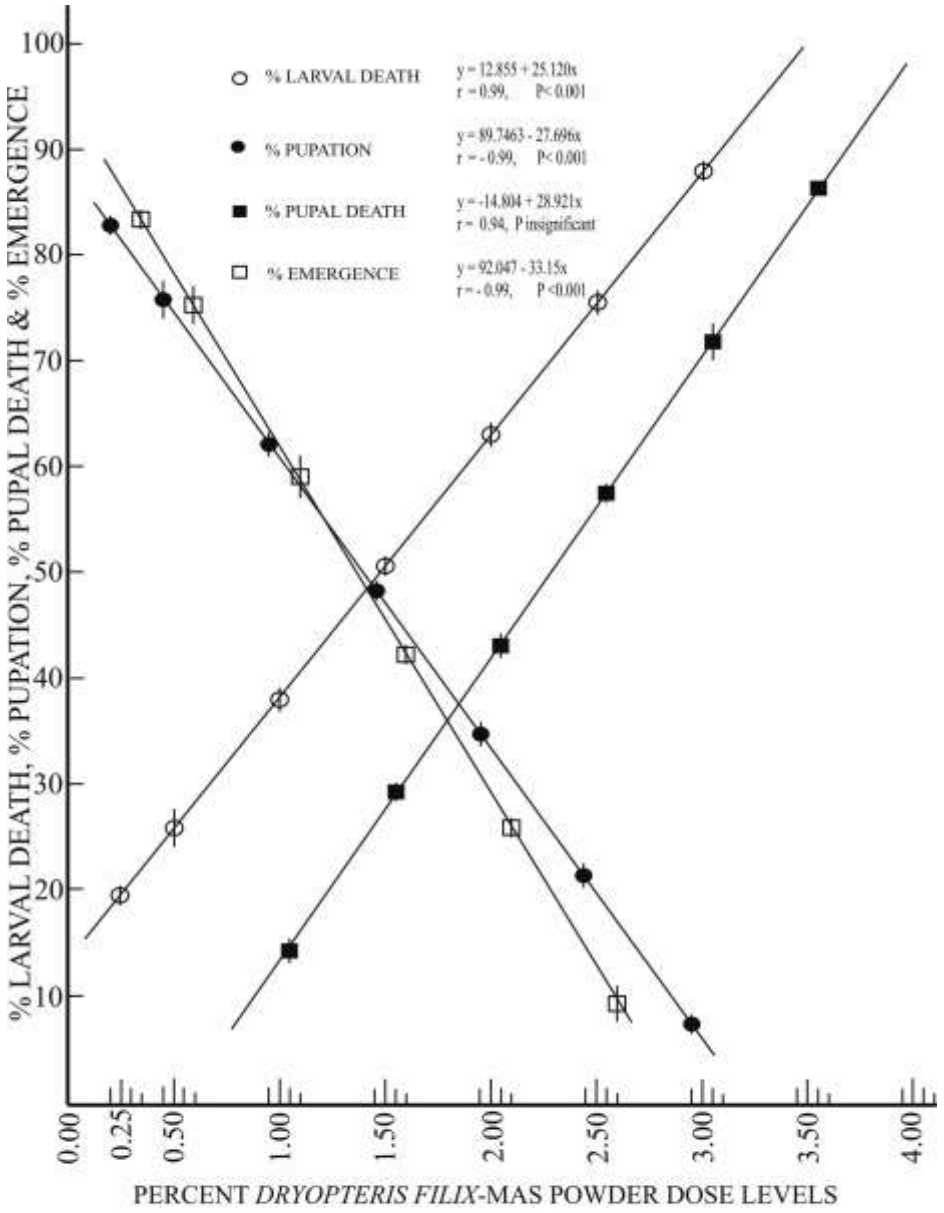

Fig. 1: Graphic representation of % larval death, % pupation, % pupal death & % emergence of rice-moth, *C. cephalonica* at various dose levels of *Dryopteris filix*-mas root and rhizome's powder (mean± S.D.).

Discussion

Plants are traditionally used in the tropics to protect stored products against various insect pests (Golob and Webly, 1980). The present investigation revealed that different doses of *Dryopteris filix-mas* root and rhizome powder exerted a depressive effect on the life cycle stages of *Corcyra cephalonica*. The toxicity of this compound increases with the increase in their doses on each developmental stages i.e. larva, pupa and adults (Table2, Fig.1). On the basis of % larval death, % pupation, % pupal death and % adult emergence, at different doses of *Dryopteris filix-mas* root and rhizome powder it is possible to categorize the relative effectiveness of their dose levels (Fitzpatrick and Dowell, 1981). The data demonstrate that 4 % dose level of *Dryopteris filix-mas* powder may be considered as extremely toxic to the pest, as no pupation occurred at

this dose level indicating 100% larval mortality. At dose level of 3.50% *Dryopteris filix-mas* pupation took place but there was no emergence of any single adult. This dose level is considered as extremely severe. At 3.00, 2.50 and 2.00% doses of *Dryopteris filix-mas* the average emergence was 2.0 ± 3.60%, 5.0 ± 3.35% and 21 ±1.80% respectively. These doses are regarded to be severely toxic. A moderately severe toxicity is accounted at doses of 1.50% and 1.00% of this compound as the average emergence at these doses was 37 ± 1.80% and 56 ± 4.00% respectively. At 0.50% dose level of *Dryopteris filix-mas* the average emergence was 74 ± 3.60%. This dose levels is considered to be moderately toxic to the pest. At dose level of 0.25% of *Dryopteris filix-mas* the average emergence was 85 ± 1.80. This dose level is considered to be poorly toxic to the pest.

The insecticidal activity of botanicals like C*alotropis gigantea* and *Ipomoea carnea* are known due to presence of flavonoids like chrysin, apigenin, morin, quercetin and myricetin (Chaterjee and Pakrashi, 1995). These flavonoids are known to inhibit some of the enzyme systems and subsequent mortality (Kuroyanagi *et al.*, 1999 and Padmavati and Reddy, 1999). Ocimum sanctum had also been known to contain linalool (3 , 7–dimethyl-1, 6 octadien-3-ol), an oxygenated monoterpenoid, which acts as a reversible competitive inhibitor of acetylcholine esterase (Ryan and Byrne, 1988). Other relevant researches reveal that extract of seeds of *Annona squamosa*, aerial parts of *Tephrosia purpurea* and rhizome of *Acorus calamus* caused toxic effects to the larvae of *C. cephalonica*, the larvae became black and resulted in to death (Jadhav, 2009). Saxena *et al.*(1992), Bhattacharya (1993), Senguttuvan *et al.* (1995), Saxena *et al. (*1996) and Patel *et al.*(1997) have also reported the effect of different plant extracts on insect pests and found several to be toxic to different insects.

Filicin present in the root and rhizome of *Dryopteris filix-mas* is a potential insecticide and is effective in arresting embryonic development in insects (Mannan *et al*., 2008). It deserves to mention that plant materials with insecticidal properties have poisonous and repellent effect and can work as phagos restrainer, ovicide and can affect the insect hormonal system (Hill, 1990). The findings of the present investigation are in accordance to earlier investigator who reported effective results with treatment of *Calotropis gigantea*, *Ocimum sanctum, Iponoea fistulosa* and *Ipomoea carnea against Sitophilus oryzae* (Srinivasan *et al*., 2003; Ryan and Byrne, 1988; Weaver *et al*., 1991; Chowdhary *et al*., 1997; Larshini *et al*., 1997; Pari *et al*., 1998; Solanki and Chitra Shanker, 2001; Chaterjee and Pakrashi, 1995; Kuroyanagi *et al*., 1999 and Padmavati and Reddy, 1999).

In the present study it appears that filicin present in the root and rhizome of *D. filix*-mas inhibits some of the enzymes system and / or release hormonal systems and subsequent mortality in rice-moth, *C.cephalonica*. It was also observed that 4% dose of *D. filix*-mas caused 100% larval mortality and hence, this dose level may be recommended for the effective control of *C. cephalonica* in particular and lepidopterous pests in general.

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RAPD-PCR based biomarker study for differentiation of store grain insects (Order: Coleoptera)

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Abstract : Random amplified polymorphic DNA (RAPD) analysis of three store grain insects *Callosobruchus maculatus, Tribolium casteneum and Rhyzopertha dominica* conducted to determine their genetic relationships. For RAPD– PCR approach, 10 primers were used. Out of them eight decanucleotide primers P1 (5'-GATGACCGCC-3'), P2 (5'-GGCACCATTC-3'), P3 (5'-GGCACGTAAC-3'), P4 (5'-GGCATGACCT-3') P6 (5'-GGTGCGCCTT-3'), P7 (*5'-GTCAGAGTCG-3'), P8* (*5'-GTCGCCGTCT-3') and P9 (5'-GTGCCAAATG-3')* were selected. The number and size of amplified products varied depending upon the sequence of random primers and genotypes used, a total of 187 discrete amplified products were obtained (size ~ 200 to 1500 bp). Out of 187 products, 118 were considered as species specific markers indicating high level polymorphism among species. It is interesting to note that *Callosobruchus maculatus* female produce one specific band in addition to male and other two species with primer P9. This specific band may be considered as a sex specific marker for *Callosobruchus maculatus*. RAPD marker can thus be successfully applied for molecular characterization and relationships in the stored grain beetles. The data obtained out of this study may be used for identification and the treatment of grains by a particular insecticide and also play a very important role in the conservation of biodiversity.

Key words: RAPD-PCR, Biomarker characterization, Stored grain insects

INTRODUCTION:

Insect comprise the largest species composition in the entire animal kingdom. Almost three-fourth of more than 8,00,000 living animal species belongs to the class Insecta. Insects are major pest of our food crops, act as vector for transmitting deadly disease, cause great damage of our urban infrastructure, environment & forest and natural resources. Insect pests in India cause an average loss of 30% in pulses valued at $ 815 million, which at times can be 100 % (Dhaliwal and Arora 1994). In general, the estimates of yield losses vary from 5 to10% in the temperate regions and 50 to 100% in the tropics (van Emden *et al.*, 1988). Their control is one of the vital factors which can improve production and storage of food.

The study of insect ecology is important to understand their evolution and diversification, and their influence on the functional and tropical links between different components of associated habitats (Speight *et al.*, 2005). In insects, DNA markers are used to provide raw information, based on which an ecologist make estimates of genetic diversity and gene flow between species (Bahura *et al.*, 2001). The greater level of polymorphism may be obtained by using DNA markers than by using protein markers (Richardson *et al.*, 1986). In molecular markers, RAPD-PCR is a conceptually simple technique for estimation of genetic diversity of insects (Williams *et al.*, 1990; Welsh *et al.*, 1990). RAPD analysis has been widely employed in evaluating genetic distances in many diverse genera. The RAPD technique has several advantages such as the ease and rapidity of analysis, a relatively low cost, availability of a large number of primers and the requirement of a very small amount of DNA for analysis (Williams *et al.*, 1990).

The objective of the present study is to analyze genetic variation between three different store grain beetles, *Callosobruchus maculatus* (cowpea weevil), *Tribolium casteneum* (red flour beetle) and *Rhyzopertha dominica* (lasser grain borer) using RAPD markers, to obtained the species-specific markers.

MATERIAL AND METHODS

Three stored grain pest *Callosobruchus maculatus, Tribolium casteneum* (Red flour beetle) and *Rhyzopertha dominica* (Lasser grain borer) were used in this study. A culture of these pests collected from the infested seeds procured from the local market of Sagar region (Central India) and reared in the laboratory (20-25°C) on sterilized seeds in glass containers, covered with muslin cloth.

DNA extraction

The genomic DNA was isolated by adult individuals of both the sexes of each species by phenol-chloroform-isoamyl alcohol precipitation method.

A single individual was homogenized in 500µl of lysis buffer. Add 30µl of proteinase K and incubate at 37°C over night. Add 500µl phenol: Chloroform: Isoamylalcohol (IAA)-25:24:1 and mix it gently by inverting the tubes for 20-25 times, spin it down for 10 minutes at 8000 rpm. After removing supernatant, add 1/10 3M Sodium acetate (50µl) and 0.8th volume of Isopropanol (400µl). Mix gently to allow DNA to clump. Spin it down for 10 minutes at 10000 rpm at 4°C. Discard supernatant, add 500µl of 70% ethanol, keep it for 10 min and spin down at 12000 rpm for 10 min at 4°C. Discard supernatant and allow the pellet to dry at room temperature or at 37°C under laminar hood. Resuspend the pellet in 60-70 µl of 10M Tris EDTA. Pellets were dissolved in water bath (55°C) overnight and store at 4°C or at -20°C for further use. These DNAs were used as templates in a PCR based search for segregating RAPD markers. The concentration of DNA was determined by UV spectrophotometer (2100/2100 Cole Parmer Ins. Company, US).

DNA amplification by PCR

The DNA was amplified by using eight decanucleotides primers with random sequences (Bangalore Genie, India).

The 50 µl of reaction mixture composed of sterile water 39.0 µl, 10X Taq Buffer A 5.0 µl, 10mM dNTP mix 2.0 µl, RAPD primer 2.0 µl, DNA template (10ng/µl) 1.0µl, Taq DNA polymerase (3 U/µl) 1.0µl. The amplification was carried out in thermal-cycler (Techne, UK) under the PCR conditions summarized in Table 2.

Table 1 – Sequences of the primers used.

Primers	**Sequences**
P1	5'-GATGACCGCC -3'
P2	5'-GGCACCATTC -3'
P3	5'-GGCACGTAAC -3'
P4	5'-GGCATGACCT -3'
P6	5'-GGTGCGCCTT-3'
P7	5'-GTCAGAGTCG -3'
P8	5'-GTCGCCGTCT -3'
P9	5'-GTGCCAAATG -3'

OBSERVATION

RAPD- PCR amplification of DNA from three stored grain pest yield series of discrete bands with eight primer P1, P2, P3, P4, P6, P7, P8 and P9 (Table1). The different primers resulted in different banding patterns because RAPD reactions often produce a pattern of bright bands together with fainter bands or faintly smeared regions in the gel. Complex pattern of faint bands can be difficult to compare among three species. Furthermore, since faint bands, especially those of higher molecular weight, exhibit inconsistent amplification from the same sample, we considered only those bands which were bright, distinct, and likely to be reproducible. Primers which produced complex or poorly resolved banding patterns were not characterized further, even if the patterns were quite different between the three species. RAPD patterns were visually analyzed and scored from photographs.

Table 2 – Cycling conditions of Polymerase Chain Reaction.

94°C	94°C	35°C	72°C	94°C	37°C	72°C	72°C
5.0 min	45 sec	1 min	1.5 min	45 sec	45 sec	1 min	10.0 min
Denaturation	X **10** cycles			X **40** cycles			Final extension

The amplified products were run on 2% agarose gel (stained with ethidium bromide) with DNA ladder (100 bp). Gels were photographed under gel documentation (MultiDoc-lt, Labmate, USA).

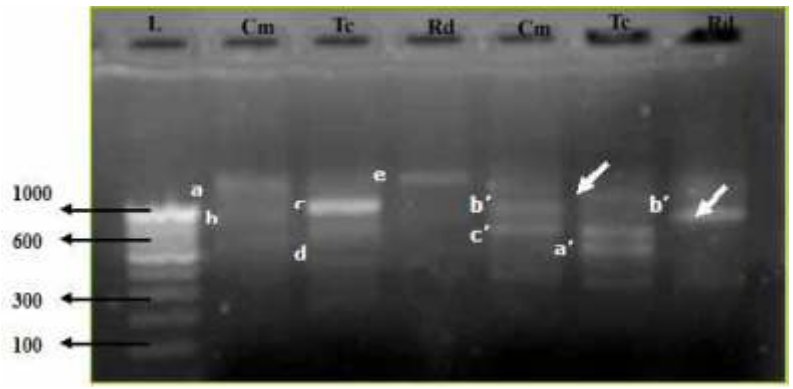

Fig. 1: Agarose gel showing bands of amplified products with Primer-P1 & P8. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* (♂). **Lane 3:-** *Tribolium casteneum* (♂). **Lane 4:-** *Rhyzopertha dominica* **(♂). Lane 5:-** *Callosobruchus maculatus* (♂). **Lane 6:-** *Tribolium casteneum* (♂). **Lane 7:-** *Rhyzopertha dominica* (♂).

Fig.1 Primer P1 & P8: Template DNA of male individuals of three species considered. Two primers **P1** and **P8** approached respectively, poor and smeared bands obtained. Primer P1 gives bright and distinct band **(>1000bp)** for *Tribolium casteneum*. Other bands not considered for comparative study. P8 showed homology between *C. maculatus* and *R. dominica* on the basis of band **b' (1000bp).**

Fig.2 Primer P2: P2 produced number of diagnostic as well as species specific pattern of bands with high intensity. Band **a** (950 bp) and **c (550 bp)** obtained with *C. maculatus*. A bright band **e (>1000 bp)** present in *T. casteneum* and bands **j (750 bp)** and **k (400 bp)** form a distinct group for *R. dominica*.

Fig.3 Primer P3:-This primer produced several diagnostic bands. Band **a (650 bp)** and **b (350 bp)** are unique for *C. maculatus*. Primer P3 provide high intensity band **c (>1000 bp)** and **f (500 bp)** for *T. casteneum*. Bands **g (800 bp)** is seen only in *R. dominica* and considered as species specific marker. Band **d (1000 bp)** and **e (950 bp)** are common in *R. dominica* & *T. casteneum* showing species specific similarity and genetic relatedness.

Fig.4 Primer P4:- This primer amplified well characterized band pattern for all three species. Many bands are common. Band **e (600 bp)** found in *C. maculatus* and *R. dominica* which is fairly bright and band **f (500bp)** shared by both *C. maculatus* and *T. casteneum* showing homology on the particular locus with phylogenetic relationship. Band **a (850 bp)** is diagnostic marker for *C. maculatus* while **b (800 bp)** and **c (300 bp)** for *T. casteneum*.

Fig. 5 Primer P6:- This primer produced three diagnostic bands. Band **a (950 bp)** and **b (700 bp)** seen with *C. maculatus* while **c (>1000 bp)** seen with *R. dominica*. The bands grouped under **d (400-800 bp)**

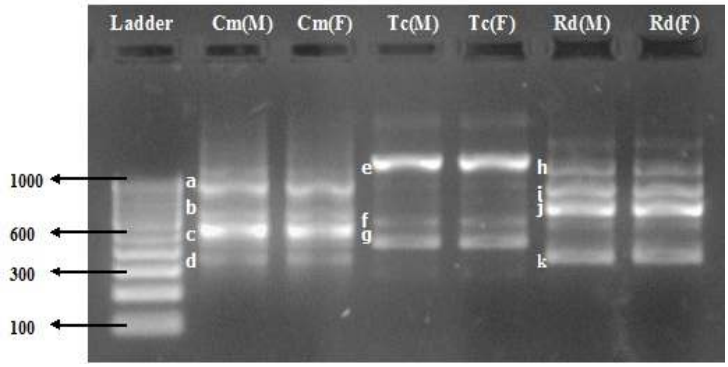

Fig. 2: Agarose gel showing bands of amplified products with Primer-P2. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* (♂). **Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂)**. **Lane 5:-** *Tribolium casteneum* **(♀). Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

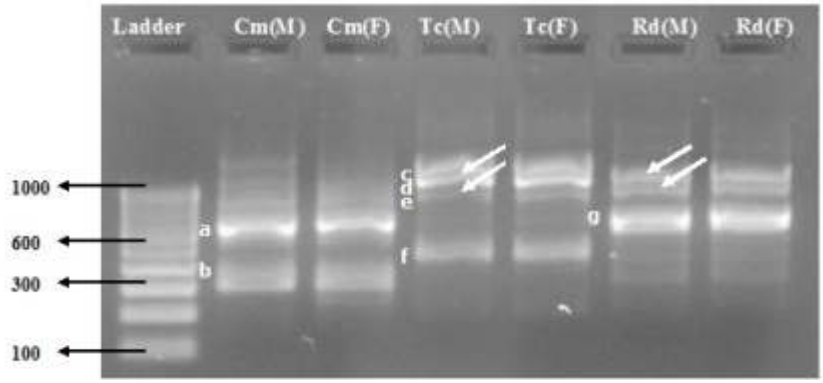

Fig. 3: Agarose gel showing bands of amplified products with Primer-P3. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* **(♂). Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂). Lane 5:-** *Tribolium casteneum* **(♀). Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

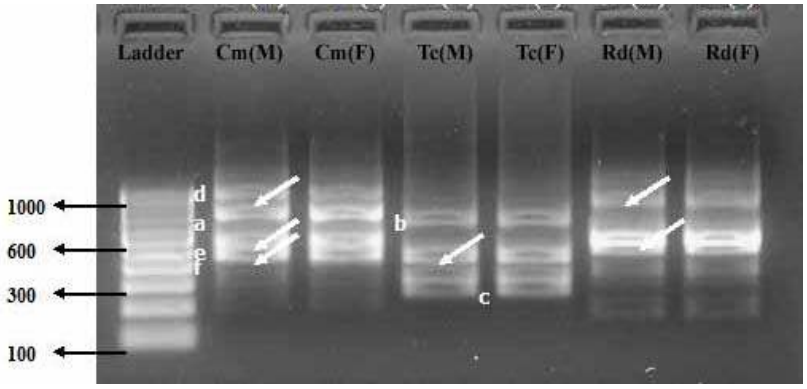

Fig. 4: Agarose gel showing bands of amplified products with Primer-P4.. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* **(♂). Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂). Lane 5:-** *Tribolium casteneum* **(♀) Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

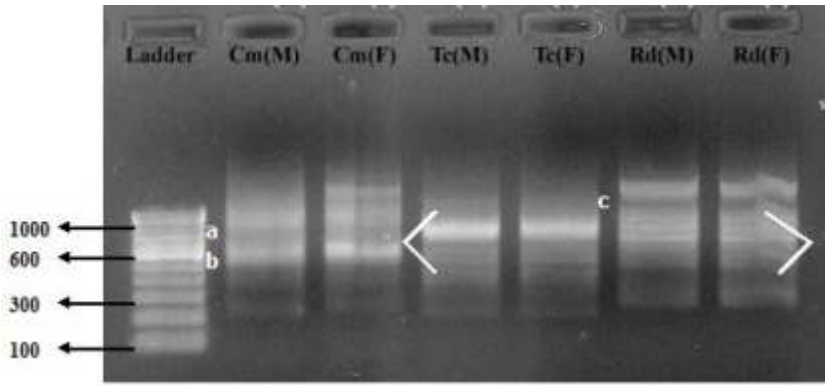

Fig. 5: Agarose gel showing bands of amplified products with Primer-P6.. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* **(♂). Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂). Lane 5:-** *Tribolium casteneum* **(♀). Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

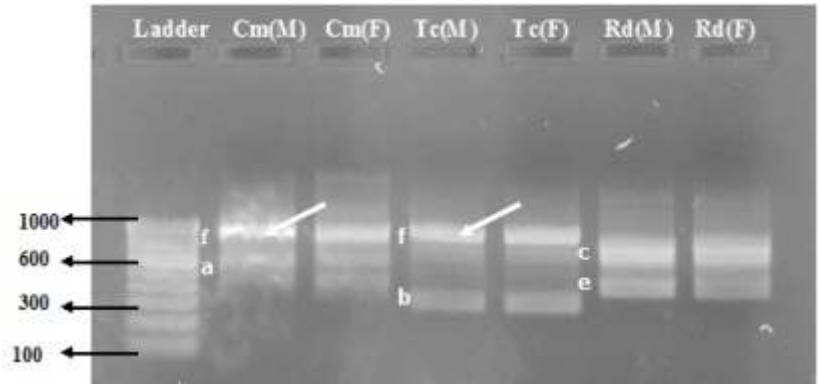

Fig. 6: Agarose gel showing bands of amplified products with Primer-P7. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* **(♂). Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂). Lane 5:-** *Tribolium casteneum* **(♀). Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

exhibited complex pattern for which homologous bands can not reliably be assigned in *T. casteneum* and *R. dominica*.

Fig. 6 Primer P7:- P7 primer produce dull and mixed but scorable bands for all three species. Variability counted as band **a (600 bp)** for *C. maculatus*, **b (350 bp)** for *T. casteneum* and **e (400 bp)** for *R. dominica*. Band **c (700 bp)** and **a' (600 bp)** in *R. dominica* appeared as a mixed band but distinct in others when run for longer periods. Homology observed by band **f (950 bp)** which is uniformly present in *C. maculatus* and *T. casteneum*.

Fig. 7 Primer P9:- P9 provides completely distinct bands for all three species. It is interesting to note that *C. maculatus* female gives a specific band **b** (**400 bp)** as compared to male and other two species. Band **a (850 bp)** for *C. maculatus*, **c (650 bp)** and **d (500 bp**) for *T. casteneum*, **e (1000 bp**), and **f (550 bp)** for *R. dominica* are scorable as species specific markers exhibited genetic differentiation among three species.

RESULTS & DISCUSSION

In the present investigation, RAPD-PCR technique applied for the molecular diagnosis of three stored grain beetles. The technique proved to be an efficient mean to obtain diagnostic molecular marker for three different stored grain beetles *Callosobruchus maculatus*, *Tribolium casteneum* and *Rhyzopertha dominica* by using eight primers that produce band patterns indicating potential to serve as a marker. The frequency of diagnostic primers observed during the present survey is quite high in comparison to other species. A number of species specific bands were

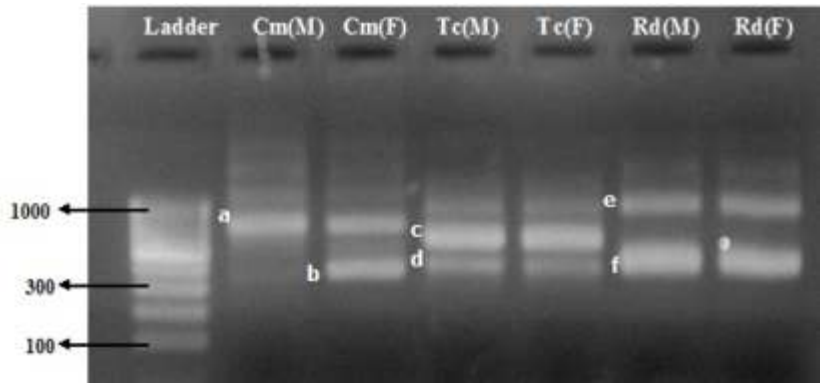

Fig. 7: Agarose gel showing bands of amplified products with Primer-P9. **Lane 1:-** 100 bp DNA ladder marker. **Lane 2:-** *Callosobruchus maculatus* **(♂). Lane 3:-** *Callosobruchus maculatus* **(♀). Lane 4:-** *Tribolium casteneum* **(♂). Lane 5:-** *Tribolium casteneum* **(♀). Lane 6:-** *Rhyzopertha dominica* **(♂). Lane 7:-** *Rhyzopertha dominica* **(♀).**

observed within RAPD profiles produced by using these primers.

Similarly Hidayat *et al.* (1996) confirmed the identity of 2 distinct species *Sitophilus oryzae* (rice weevil) and *S. zeamis* (maize weevil) through their RAPD-PCR pattern. The RAPD-PCR technique has been proved to be useful in the population genetics studies of mosquitoes (Kambhampati *et al.*, 1992), *Diapreps abbreviatus* (Coleoptera) (Bas *et al.*, 2000), honey bees *Apis mellifera* (Sheppard and Smith, 2000) and in various other insect populations. In the present study for each primer evaluated, a multiple band profile comprising one to four major amplification products and a varying number of weak products or a faintly smeared region was observed. The number and size of amplified products varied depending upon the sequence of random primers and genotypes used; a total of 187 discrete amplified products were obtained ranging from 200 to *1500* bp. Out of which, 118 were counted as species specific markers showing high level polymorphism (Table 3).

All the primers in the species studied, amplified the comman bands suggesting the intraspecific genetic relatedness. There were, however, some bands specific to both the sexes. These specific bands showed genetic variations in the two sexes. Species specific bands were also observed which revealed the existence of some conserved regions within the species. These conserved regions provided diagnostic profiles for these species. Such diagnostic markers have also been reported in three species of white-fringed weevils (Coleoptera) and two of *Parnassius* (Lepidoptera: Papilionidae) by Hardwick *et al* (1997) and Zakharov (2001) respectively. Primers P6 and P9 in the species studied, amplified the male and female specific band suggesting the genetic variation in the two sexes.

The observations obtained out of RAPD-PCR analysis revealed that three stored grain beetles *Callosobruchus maculatus, Tribolium casteneum and Rhyzopertha dominica* in the population are quite distinct at genetic level. The greater genetic variability of natural populations will complicate the search for completely diagnostic primers, but the minimal effort of screening additional primers suggests that this will not be an insurmountable problem. The ability of RAPD analysis to detect polymorphism holds great promise for detecting population structure and genetic differentiation even within well established 'species' that

Table 3:- Number of amplified bands and no. of selected species specific bands with eight Primers in three species of stored grain pests [*Callosobruchus maculatus* (C.m.), *Tribolium casteneum* (T.c.) and *Rhyzopertha dominica* (R.d.)].

Primer	Nucleotide	Size range of amplified bands (bp)	Total number of bands						Number of selected species-specific RAPD bands					
			C.m.	*C.m.* ♀	*T.c.* ♂	*T.c.* ♀	*R.d.* ♂	*R.d.* ♀	*C.m.* ♂	*C.m.* ♀	*T.c.* ♂	*T.c.* ♀	*R.d.* ♂	*R.d.* ♀
P1	***GATGACCGCC***	500 >1000	2	-	3	-	1	-	2	-	2	-	1	-
P2	***GGCACCATTC***	300 > 1000	5	5	6	6	6	6	4	4	3	3	4	4
P3	***GGCACGTAAC***	300 >1000	5	5	4	4	6	6	2	2	4	4	3	3
P4	***GGCATGACCT***	200 >1000	6	6	3	3	5	5	4	4	3	3	2	2
P6	***GGTGCGCCTT***	200 >1000	3	4	6	6	5	5	2	2	4	4	4	4
P7	***GTCAGAGTCG***	250 >1000	5	5	4	4	5	5	2	2	2	2	3	3
P8	***GTCGCCGTCT***	250 >1000	4	-	4	-	2	-	2	-	1	-	1	-
P9	***GTGCCAAATG***	400 > 1000	4	5	4	4	3	3	2	3	3	3	3	3

would otherwise go unnoticed (Jain *et al.,* 2010).

RAPD therefore, appears to be useful technique in differentiating species, subspecies and strains in insects such as in *Aades aegyptii* (Ballinger-Crabtree *et al.*, 1992), in the identification of aphid species and clones (Cenis *et al.*, 1993), in gypsy moth (Tom *et al*., 1995), in Indian meal moth (Dowdy and Mcgaughey, 1996), in silkworm *Bombyx mori*, (Weng *et al.*, 1996), in two species of *Catopsilia* (Sharma *et al.*, 2003), for Butterflies species (Sharma *et al.*, 2006) and in four species of family Pieridae (Tiple *et al.*, 2010)

CONCLUSION

The RAPD-PCR technique is extremely useful for rapid identification of genetic polymorphisms in three stored grain beetles (*Callosobruchus maculatus, Tribolium casteneum* and *Rhyzopertha dominica*) because of the reproducibility of the results for each of the species. The bands generated using eight primers by RAPD are clear genetic markers. The technique may be used for genetic identification of other plant and animal species. However, the large data will strengthen the applicability of the technique by increasing the number of individuals and the primers for the study in future.

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Design of a SOI-MEMS Piezoelectric Accelerometer for Biomedical Applications

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Abstract-The piezoelectric micro accelerometers have wide applications in the field of auto mobiles, inertial navigation and bio medical instrumentation. The main aim of our research is to develop a piezoelectric accelerometer that can measure body movements in assessing the neurological disorders like parkinsons disease. The most important specifications for medical applications are amplitude range +/-5g. resolution 10 -3g, bandwidth d.c.-50 Hz, off axis sensitivity < 5%, low drift and power consumption < 1mW. The accelerometer design employs a proof mass suspended by four beams. When the device is accelerated the mass moves relative to the anchor, the beams deflect causing the piezoelectric layers to deform and hence a charge is induced .The amount of charge generated depends upon the piezoelectric properties of PZT material, and many dimensional design considerations. This design is proved to get very high sensitivity, good frequency response. FEM simulations are performed by the CoventorWare® to investigate the device behavior. Displacement of the structure due to the applied acceleration of 1g has been simulated. Charge sensitivity is found to be 3.201X10-6 Col/g at the resonant frequency of 58Hz has been evaluated.

Key Words: Coventorware, Piezoelectric Accelerometers, Charge Sensitivity, Off axis sensitivity, Silicon On Insulator (SOI).

Introduction

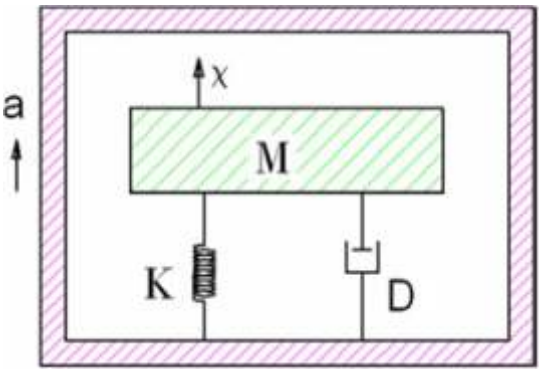
Micro electro mechanical systems which permits the integration of micro machined mechanical structures with integrated circuits, has been the growing area of research in last two decades. MEMS have a wide range of applications including automotive ,industrial, biomedical and information processing. Silicon micro machining , taking the advantages of well established processes and materials in microelectronics ,is commonly used to fabricate mechanical micro structures. There are many functional materials that can be fit with MEMS. PZT is the one of those materials that is attractive because of their high sensitivity in sensor applications. Its high electromechanical coupling and piezoelectric constant, which are an order of magnitude larger than ZnO and AlN. Accelerometers have been used in many fields including for activation of automotive safety systems, for machine and vibration monitoring and in biomedical applications for activity monitoring. Chen et al. (1982) fabricated a Silicon bulk micro machined piezoelectric accelerometer with cantilever beam structure using a ZnO film and obtained a sensitivity of 47µV/g. In 1984 Chen and Muller redesigned the same accelerometer with a huge Si mass which was 20 times their previous design and were able to obtain the sensitivity of 5mV/g with a resonant frequency of 8.4KHZ. Nemirovsky et al.(1996) designed a PZT thin film piezoelectric accelerometer(Nemirovsky et al., with a sensitivity of 320mV/g. De Voe and Pisano(1997)designed a ZnO accelerometer (Devoe et al ., 2001) which gives a low sensitivity of 0.95fC/g. Reus (1999) developed a ZnO accelerometer with single proof mass and two suspension beams with a sensitivity of 0.1pC/g and a resonant frequency of 4.5khz. Beeby (1999) developed a bulk micro machined accelerometer with a very high sensitivity of 16pC/g. In the same year 1999, Eichner designed a bulk micro machined PZT accelerometer with a single proof mass and 2 suspension beams and the sensitivity of 0.1mV/g was obtained with 13kHZ resonant frequency. Wang (2003) fabricated a bulk micro machined piezoelectric accelerometer with annular diaphragm structure and obtained a sensitivity of 0.77pC/g with resonant frequency of 3.7kHZ. Yang (2007) designed the bulk micro-machined piezoelectric accelerometer with 12 different structures. All the above accelerometer designs make use of bulk micromachining or surface micro machining with piezoelectric sensing. Every design has its own advantages and disadvantages. However we have designed an accelerometer that is

useful for biomedical applications where our main goal is to get the good sensitivity with given specifications. In order to measure physical activity accelerometers must be able to measure +/- 5g at the waist level and frequencies between 0 to 30 Hz.

Design

Piezoelectric material provides its own internal biasing requirement, either due to absence of a center of symmetry in the case of single-crystal materials such as aluminum nitride (AIN) or zinc oxide (ZnO) or due to a permanent polarization present in ferroelectric materials such lead–zirconate–titanate (PZT). A typical piezoelectric accelerometer consists of a layer of piezoelectric material sandwiched between a mounting plate and a seismic mass (Fig. 1 & Table 1). When a force or a pressure is applied to the opposite faces of the piezoelectric material, an electric charge is produced. This charge can be amplified to give an output voltage that is proportional to the applied force or acceleration.

Fig. 1. Basic Accelerometer Model



The basic equation (1) of the system is

$$M\frac{d^2x}{dt^2} + D\frac{dx}{dt} + kx = -F = -Ma$$

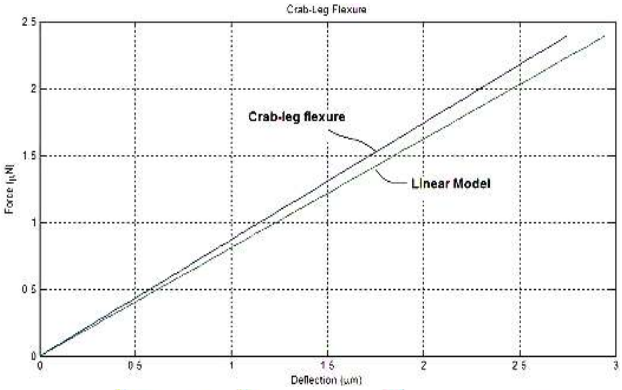
The most important characteristics of an accelerometer are the sensitivity and the operating frequency range. The sensitivity is defined as the ratio between the electrical output (charge or voltage) and the mechanical input (force or acceleration);the band where the sensitivity remains practically unchanged defines the operating frequency range which is upper limited by the first resonance frequency of the device. In the classical accelerometers, which are one-directional, the increase of the sensitivity is obtained by increasing the seismic mass. Nevertheless, the added mass produces a lowering of the resonance frequency and, therefore, a narrowing of the operating frequency range. Mechanical accelerometers consist of a spring-mass system, with a seismic mass carried by elastic tether beams.

Table 1. Specifications of Accelerometer

Band width	dc-50HZ
Amplitude range	+/- 5g
Off axis sensitivity	<5%
Power consumption	< 1mw

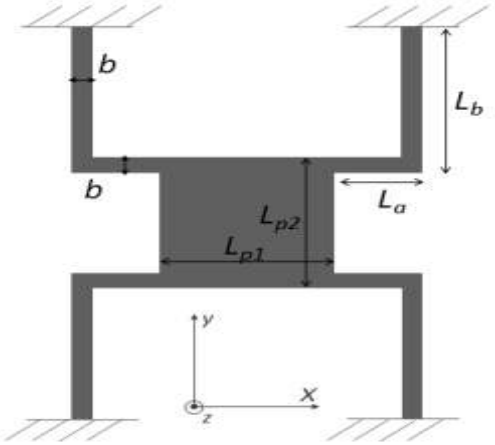
Acceleration along the sensitive axis leads to a deflection of the seismic mass, with elastic forces from the tether beams balancing the external forces. The design shown in Fig. 3 employs a proof mass with four crab leg beams. Crab leg beam consists of a added thigh section La to the beam Lb . The added thigh section, length 'La' minimizes the peak stresses in the flexure. The deflection of the thigh also reduces the extensional stresses. the stiffness 'K'of the crab-leg flexure unlike the fixed-fixed flexure, can be varied by varying the values of lengths and widths of thigh and shin segments. The crab-leg flexure has linear characteristics closely matching the linear deflection model (Abbas, 2008) for small deflections as shown in Fig. 2

Fig. 2.Deflection of the crab leg flexure Figure



$$Q = \int D.w.dl = \int d_{31}\, E_p . \frac{M}{\sum E_i (I_i + A_i h_i^2)} . z_p . w . dl$$

Fig. 3. Structure of Accelerometer



With m and k the seismic mass and the spring constant of the spring-mass system, the resonance frequency of the spring. The spring constant estimated as

$$k = \frac{\propto E_{max} b \sum \frac{E_i}{E_n} \frac{t_i^3}{12} + t_i \frac{E_i}{E_n} (h_i - h_n)^2}{l^3}$$

where α is a constant value correlated to the springs, Emax is the maximum Young's modulus of the stack materials, l is the equivalent length of the spring, given by a contribution of La and Lb, as shown in Fig.3b is the width of the beam ,hn is the neutral axis position assumed in the homogeneous domain. Ei, ti, hi represent the Young's modulus, thickness and the neutral axis position for the i th element .

Piezoelectric sensing

The charge induced on the beam is due to piezoelectric effect of PZT film deposited on the beam.The induced electric polarization due to stress is given by (Smith *et al*., 1991,Eichner *et al*.,1999).]

D= € EFIELD + d σ

Where € and d are the permittivity and piezoelectric coefficient of PZT.

For piezoelectric sensing usually electric field is not applied so

D= d σ

The normal stress according to piezoelectric relations given by M.S Weinberg (Smith et al., 1991)

$$\sigma = E_p . \frac{1}{R} . Z_p = E_p . \frac{M}{\sum E_i (I_i + A_i h_i^2)} . z_p$$

Where Ep is the youngs modulus of PZT,Ii is the moment of inertia of each layerin the beam,Ai is the cross section area of each layer,Zi is the distance between center of each layer and neutral plane of the beam.

According to Weinberg (1999) the charge produced on the electrode is

$$Q = \int D.w.dl = \int d_{31} E_p . \frac{M}{\sum E_i (I_i + A_i h_i^2)} . z_p . w . dl$$

Therefore charge sensitivity is nothing but the charge produced by unit acceleration.

Fabrication

In the past, manufacturing methods employed to make inertial MEMS devices could be roughly divided into either surface or bulk micro-machining . Newer methods involving the use of silicon on insulator (SOI) substrates are becoming increasingly popular. The use of SOI substrates combines the manufacturing advantages of surface and bulk micromachining. The other advantages of using SOI technology are feasibility of fabrication of very high aspect ratio structures by using deep reactive ion etching (DRIE), effective electrical isolation between two silicon layers by an oxide layer resulting in superior electrical performance, SOI substrates facilitate the combining of CMOS electronics and MEMS devices on the same wafer, Process complexity and costs are reduced when the SiO2 layer is used as an etch stop. Thus, SOI technology is the best choice for making devices like high performance accelerometers and gyroscopes etc. SOI substrate is formed with 450 µm Si, 2 µm SiO2 and 15 µm Crystal Si. Thermal grown oxide of 0.1 µm thickness is stacked on the SOI wafer. Reactive Ion Etching is done to form the mass and beams from the front side. PZT material of 0.7 µm is deposited on the front side by sol-gel deposition. Aluminum of 0.6 µm is deposited on the front side using PECVD technique. PZT and Aluminium are removed from front side by applying masks. 450 µm Si is removed from backside to form the beams. FEM simulations are performed by the CoventorWare® to investigate the device behavior. An example of simulation results is shown in Fig. 4 & 5. Displacement of the structure due to the applied acceleration of 1g has been simulated. Charge sensitivity is found to be 3.201X10-6 Col/g at the resonant frequency of 58Hz has been evaluated.

Table 2. Material properties of layers

Material	Young's modulus (GPa)	Density ($X10^{-15}$ kg/ µm^3)
Aluminium	77GPa	2.3
Si_iO_2	70 GPa	2.2
PZT	148 GPa	7.85
Silicon	160 GPa	2.5

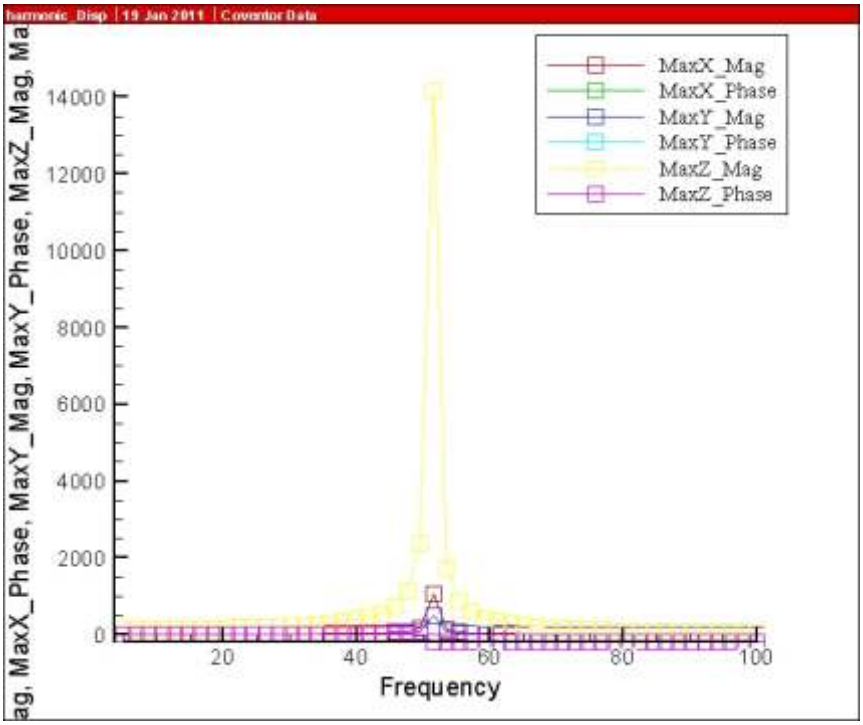


Fig. 4. Frequency Response of Accelerometer

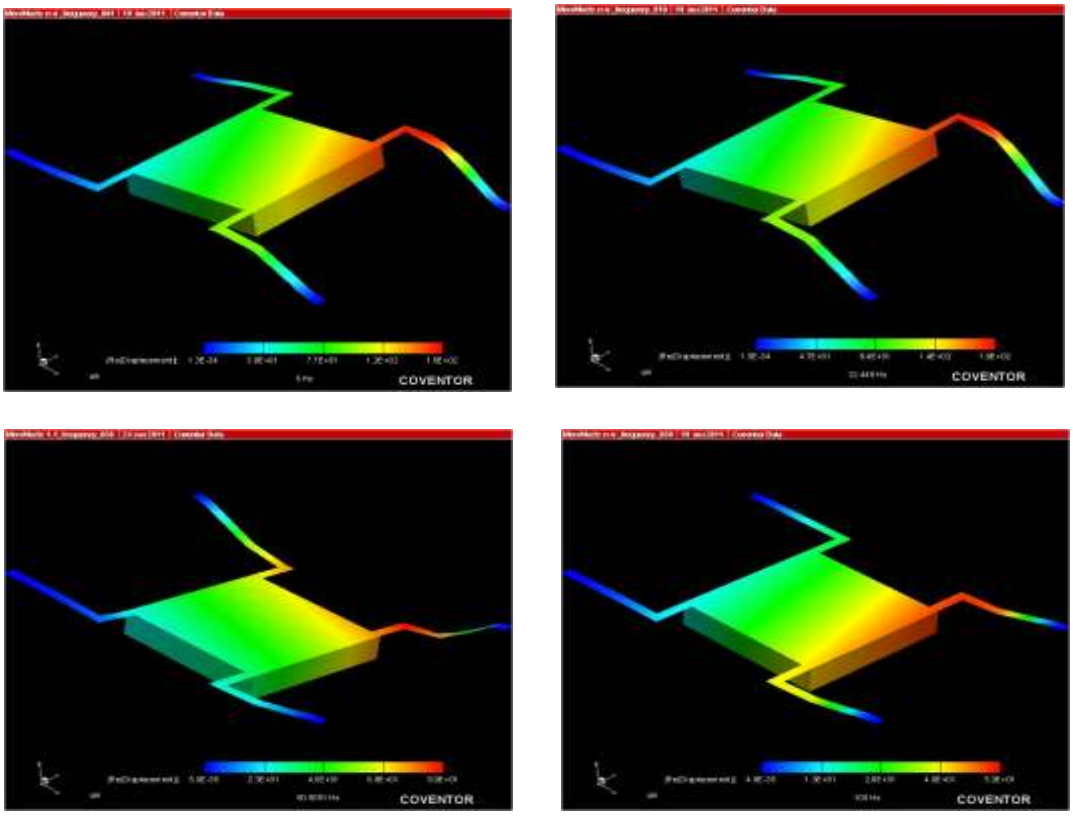


Fig. 5. Examples of results obtained by the simulation of the device behavior in Coventorware Environment a) 5Hz b) 22Hz c) 50Hz d) 100 Hz

Table 3. Designed accelerometer parameters

Proof mass length(μm)	3000
Beam length, L_a(μm)	600
Beam length, L_b(μm)	2200
Beam width(μm)	200
Beam Thickness (μm)	16.8
PZT thickness(μm)	0.7
Aluminium thickness(μm)	0.6
Thickness of proof mass(μm)	450

Results and Discussions

MEMS Piezoelectric accelerometer is designed with large proof mass using SOI technology and simulated in Coventorware environment (Table 2 & 3). Deflection of the structure at different frequencies are observed and we found very satisfactory results at low frequencies (Fig. 5) as the design is intended especially for measuring hand tremors which are at low frequencies. The unique feature of this design is its highest charge sensitivity of 3.201X10-6 Col/g at the resonant frequency of 58Hz. The present investigation is in canvas with the findings of the previous investigators (Nemirovsky et al.,1996; Reus, 1999).

Conclusion

A high sensitivity MEMS Piezoelectric accelerometer is designed and analyzed in CoventorWare® to investigate the device behavior. This design proved to get high sensitivity because of its structure, device dimensions ,and the PZT material properties. We can further increase the sensitivity by increasing proof mass dimensions but there is a trade off between proof mass and resonant frequency. The advantages of SOI technology and structure of accelerometer are exploited to achieve the high performace accelerometer useful for medical applications.

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One Step Tissue Culture Propagation of Peppermint - *MENTHA PIPERITA*, a NOVEL Technique

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Running Title - ONE STEP TISSUE CULTURE PROPAGATION OF PEPPERMINT

ABSTRACT : The present study explains the importance of rapid one step micropropagation of *M. piperita* to produce disease free propagules round the year without seasonal constraints. Shoot apex and nodal explants were surface sterilized and inoculated on MS basal medium supplemented with different concentrations of BAP/KIN. Shoot apex cultured on BAP (2.0 - 2.5 mg/l) produced callus, axiliary shoots and roots on same medium only in one step without subculture to another/differentiation medium in the second step. Generally more than two steps are required in normal tissue culture propagation. Plantlets were also differentiated from callus on the same medium in single step only. Hence, one step rapid micro-propagation protocol was developed to make the process fast and cost effective. Nodal stem culture produced less frequency of auxiliary shoots and regeneration of plantlets. Shoot apex explant was found most suitable for single step propagation. Developed plantlets were hardened and transferred to the field where 55 – 60 % plantlets survived. Rapid and cost effective micro-propagation protocol thus developed may be of significant value for commercialization of tissue culture propagation methods.

Keywords : Clonal propagation, in-vitro propagation, *Mentha piperita*

Abbreviations : BAP- 6-Benzyle Amino Purine, KIN- 6 furfurylamino purine (kinetin), LS – Linsmaier and Skoog (1962), MS- Murashige and Skoog

Introduction

The genus *Mentha* (Lamiacae) includes about 25 species that are mostly aromatic perennial herbs with creeping rhizomes and scented foliage. Several species of *Mentha* are cultivated in different parts of the world for their medicinal and commercial importance. Oil glands of mature leaves and flowers contain principal ketone, alcohol and ester compounds-called 'essential oil'. The essential oil of *Mentha piperita* (peppermint) is widely used in perfumery, food and pharmaceutical industries (Cellarova, 1992). Menthol, the most important component used in these industries, is obtained by crystallization of 'essential oil'.

The majority of *Mentha* species are multiplied vagetatively through the rhizome system. Natural hybridization occurs among different species but most of the hybrids are sterile or subfertile. Vegetative propagation enables a large number of such hybrids to establish themselves successfully along with their parents creating a taxonomic complication. *M. piperita* can be identified on the basis of chromosome no. 2X=72.

In-vitro propagation of *M. piperita* is essential because it enables the production of a large number of uniform plants for planting in a small space and within a short time interval. Sexual reproduction of *Mentha* is of no importance because progeny is usually semifertile or sterile. Tissue culture propagation also helps in elimination of various pathogens such as viruses, mildews, etc. Callus and cell culture are used as a model system for studying the biosynthesis and biotransformation of desirable secondary metabolite and its extraction (Cellarova, 1992).

Tissue culture reports of *M. piperita* available so far, are mostly related to cell suspension culture for secondary metabolite production (Stohs and Staba, 1965, Becker,1970, Kireeva *et al.*, 1978, Rodov and Reznikova, 1982, Galun *et al.*, 1985). Cellarova *et al.* (1984), Repcakova *et al.* (1986), Venkataraman and Ravishankar (1986) reported micropropagation of *Mentha piperita* by subculturing 2-3 times on MS/LS medium supplemented with different combinations of growth adjuvants. Micropropagation by repeated subculturing is time, money and space consuming technique. This communication presents a highly efficient, rapid and cost effective method for clonal

multiplication of *M. piperita* to be performed in single step without going to subculture steps.

Materials and Methods

Shoot apices with 2-3 nodes and nodal explant from 3-5th node from shoot tip of mature plant were taken and washed properly with plenty of tap water. Then it was kept in teepol for 10 minutes and again washed in running tap water until the detergent is completely washed out. It was then treated with 70% alcohol for one minute and surface sterilized in 1% mercuric chloride ($HgCl_2$) for 2.5 minutes. Finally the explants were washed with sterile distilled water 3-4 times and inoculated in culture tubes (15cm × 2.5cm)/ in 150ml Erlenmyer flasks containing culture medium over the flame of spirit lamp inside the laminar flow. The cultures were maintained at 16 hour photoperiod, temperature 23-28ºC, relative humidity 70% and illuminance 2500 Lx. Murashige & Skoog (1962) (MS) medium with 8 gm/I agar was used as basal medium. The basal medium was supplemented with different concentrations of cytokinins - BAP (6 - benzyle aminopurine) / KIN (6 - furfuryl amino purine). After the development of shoots, bottom of the tubes/flasks was covered with black paper to maintain continuous darkness suitable for root development. Each treatment had 10 replicates of cultures in test tubes and/or 5 replicates of cultures in flasks. *In-vitro* developed plantlets of size 4-6 cms were taken out, washed properly and transferred to a porous basket with sterilized sand. The plantlets were covered with ventilated polythene film and kept in high humidity (60-70%). Initially it was watered with MS inorganic nutrients 3-4 times a day. Gradually it was acclimatized for field condition.

Results

Shoot apex culture:

MS basal medium with BAP at 1.5-3.0 mg/I produced green compact callus showing moderate growth. However, 2.0 mg/l BAP produced fast growing callus. Basal medium with BAP (2.0 mg/l) produced axiliary shoots in 91.5% of shoot apex cultures and 17.5 shoots per culture were found after 2-3 weeks (Fig. 1&2). These micro-shoots when attained a length of 4.5 cm, 80.3% of them rooted on the same medium without sub-culturing on rooting medium (Table-1). 82.5% of cultures which developed callus showed regeneration of shoots after 4 weeks (Fig.3) and later, after 5 weeks roots also developed in the regenerated shoots on the same medium (Table-3, Fig. 4) BAP was found more suitable than KIN (Table 1 & 2). Complete plantlets were produced in one step without transferring to the rooting medium (Fig. 4 & 5). Almost similar result was found on BAP (2.5 mg/l) with less frequency and less number of shoots/plantlets per explant. Secondary and tertiary axillary shoots were also developed. BAP (2.0 mg/l) was found most suitable for shoot/root/plantlet regeneration directly from shoot-apex as well as differentiation of plantlets from callus (Table-1 & 3). This concentration of BAP induced 17.5 microshoots per explant (shoot apex) and 4.5 roots per micro-shoot. After 6-7 weeks interval vigorous rooting was found. Average length of shoot was 5.2 cms with maximum 16.3 cms and minimum 2.5 cms of length. Average length of root was 3.2 cms with maximum 8.2 and minimum 1.5 cms. However, same concentration of BAP induced differentiation of 35.2 plantlets per callus culture having 4.9 roots per shoot. Average length of differentiated shoot was 5.6 cm and that of root was 3.1 cm (Table-3).

KIN (1.0-2.0 mg/l) was found less effective for shoot/root/plantel regeneration directly from shoot-apex or through callus as compared to BAP (Table-1 & 3).

Nodal stem culture:

BAP (1.5-3.0 mg/l) produced compact green callus while KIN (1.0-2.0 mg/l) produced the same but in less frequency. KIN (1.0-2.0 mg/l) and BAP (1.5-3.0 mg/l) induced regeneration of microshoots in nodal explants but was less effective as compared to shoot-apex explant (Table 1 & 2). BAP at 2.0 mg/l and KIN at 1.0 mg/l were found most suitable for plantlet regeneration (Table-2).

Shoot apex was found the most suitable explant and BAP (2.0 mg/l) was found the most suitable hormone for one step clonal propagation of M. piperita. The plantlets thus formed were acclimatized and transferred to the field where 55-60% plantlets survived (Fig. 6&7).

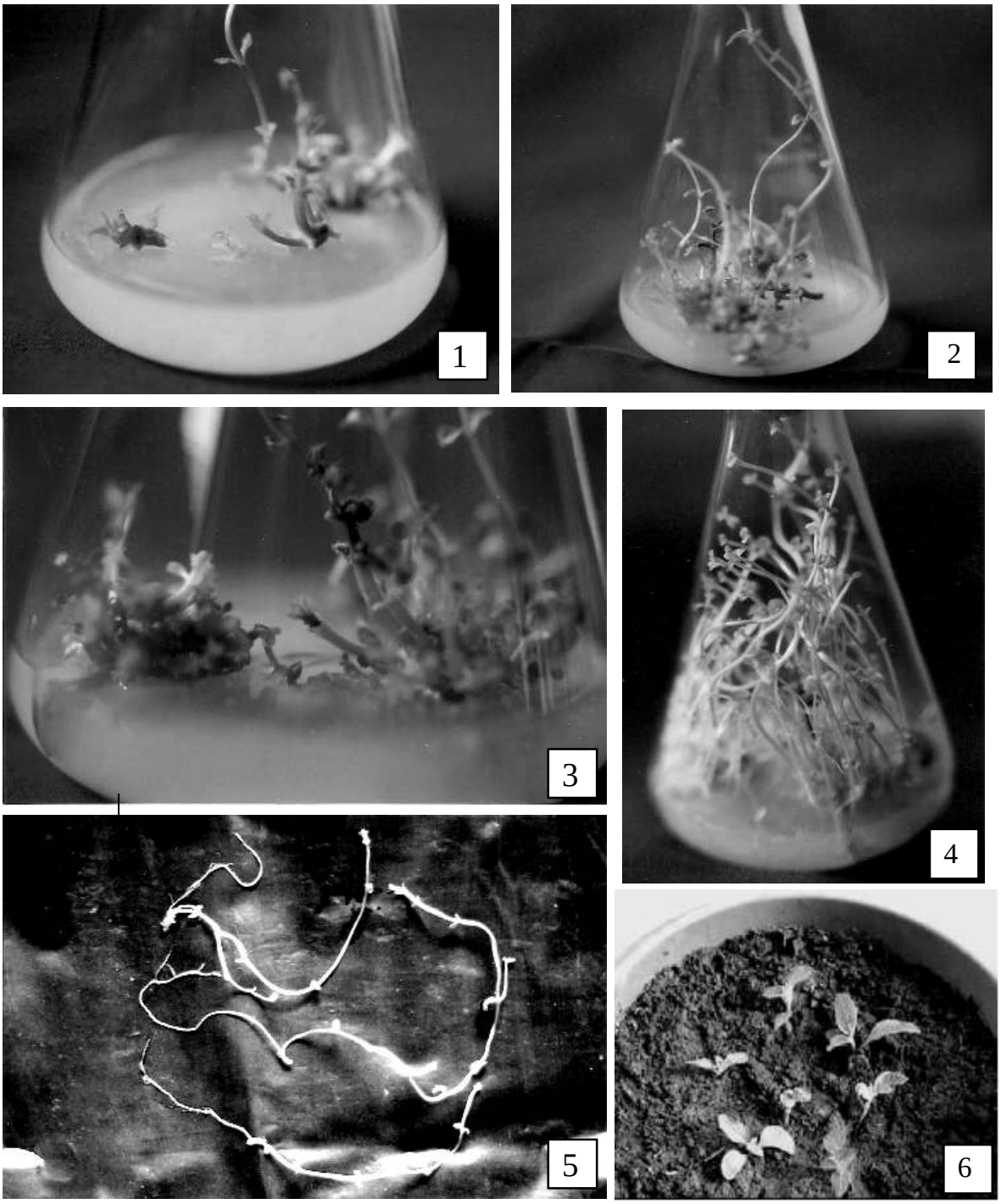


Figure.1-6. One step *in-vitro* propagation of *Mentha piperita*

1. Regeneration of micro shoots in shoot-apex explants after 2 weeks of culture on MS medium with BAP (2mg/l) (×1.14)
2. Multiple shoot proliferation and callus formation in shoot-apex explants after 3 weeks of culture on same medium (×0.7)
3. Differentiation of multiple shoots from callus after 4 weeks of culture on same medium (×1.7)
4. Shoot elongation and differentiation of roots in shoots after 5-6 weeks of culture on same medium (×0.8)
5. Differentiated plantlets with well developed shoot and root system after 6-7 weeks of culture (×1.8)
6. Acclimatization of plantlets in green house.

Table-1.Single step regeneration of plantlets from shoot-apex of *Mentha piperita* cultured on MS medium supplemented with Benzyle Amino Purine (BAP) / 6 furfuryl amino purine (Kinetin/KIN)

Treatment (mg/l)	Mean no. of regenerated microshoots per explant ± SE	Percentage of microshoots rooted	Mean no. of roots per microshoot ± SE	Mean length of shoot (root) per plantlet cm ± SE
BAP				
0.0 (Control)	1.2 ± 0.2	15.3	1.8 ± 0.5	1.8 ± 0.4 (0.8 ± 0.2)
1.5	15.2 ± 0.8	82.4	3.8 ± 1.3	2.2 ± 0.8 (1.3 ± 0.3)
2.0	17.5 ± 1.6	80.3	4.5 ± 0.3	5.2 ± 1.2 (3.2 ± 1.4)
2.5	10.3 ± 2.5	65.2	3.5 ± 0.8	3.2 ± 0.4 (1.8 ± 0.6)
3.0	5.1 ± 2.6	45.3	2.2 ± 1.2	2.5 ± 0.8 (1.2 ± 0.6)
KIN				
1.0	6.3 ± 1.4	48.3	2.3 ± 0.8	2.2 ± 0.2 (1.2 ± 0.6)
1.5	4.3 ± 1.8	35.6	3.2 ± 0.7	3.8 ± 0.4 (1.5 ± 0.6)
2.0	1.8 ± 0.6	19.8	2.1 ± 0.6	2.1 ± 0.2 (1.4 ± 0.4)

Results noted after 6-8 weeks of culture, MS = Murashige & Skoog (1962), SE = Standard error

Table -2.Single step regeneration of plantlets from nodal stem of *Mentha piperita* cultured on MS medium supplemented with Benzyle Amino Purine (BAP) / 6 furfuryl amino purine (Kinetin/KIN)

Treatment (mg/l)	Mean no. of regenerated microshoots per explant ± SE	Percentage of microshoots rooted	Mean no. of roots per microshoot ± SE	Mean length of shoot (root) per plantlet cm ± SE
BAP				
0.0 (Control)	1.5 ± 0.4	12	1.8 ± 0.2	1.5 ± 0.6 (0.8 ± 0.2)
1.5	6.2 ± 1.2	17	2.8 ± 0.4	4.5 ± 0.6 (2.1 ± 0.4)
2.0	8.3 ± 0.4	22	2.1 ± 0.7	3.2 ± 0.8 (1.8 ± 0.9)
2.5	7.4 ± 1.2	20	1.9 ± 0.6	2.8 ± 0.6 (1.6 ± 0.4)
3.0	3.1 ± 0.4	18	1.5 ± 0.4	2.5 ± 0.5 (1.3 ± 0.5)
KIN				
1.0	5.2 ± 0.6	14	1.8 ± 0.4	2.3 ± 0.2 (1.2 ± 0.2)
1.5	3.3 ± 0.9	15	2.1 ± 0.3	3.2 ± 0.5 (1.5 ± 0.8)
2.0	2.1 ± 0.8	13	1.5 ± 0.8	2.8 ± 0.8 (1.3 ± 0.6)

Results noted after 6-8 weeks of culture, MS = Murashige & Skoog (1962), SE = Standard error

Table -3.One step differentiation of plantlets from callus produced from shoot-apex of Mentha piperita cultured on MS medium supplemented with Benzyle Amino Purine (BAP)

Treatment (mg/l)	Nature, colour, Growth rate of Callus Produced	Mean No. of differentiated Plantlets per culture ± SE	Mean no. of differentiated roots per plantlet ± SE	Mean length of shoot (root) per plantlet cm ± SE
BAP				
0.0 (Control)	-----	00	00	00
1.5	FG++	15.5	3.5 + 0.4	3.2 + 0.4 (1.3 + 0.7)
2.0	CG+++	35.2	4.9 + 1.2	5.6 + 0.7 (3.1 + 0.4)
2.5	CG++	17.7	3.2 + 0.8	2.9 + 0.5 (1.8 + 0.9)
3.0	CG+	12.5	2.1 + 0.4	2.4+ 0.3 (1.2 + 0.4)

Results noted after 6-8 weeks of culture, MS = Murashige & Skoog (1962), SE = Standard error, F=Friable, C=Compact, G=Green, +=Slow, ++=Average, +++= Fast.

Fig. 7. Field grown plants.

Discussion

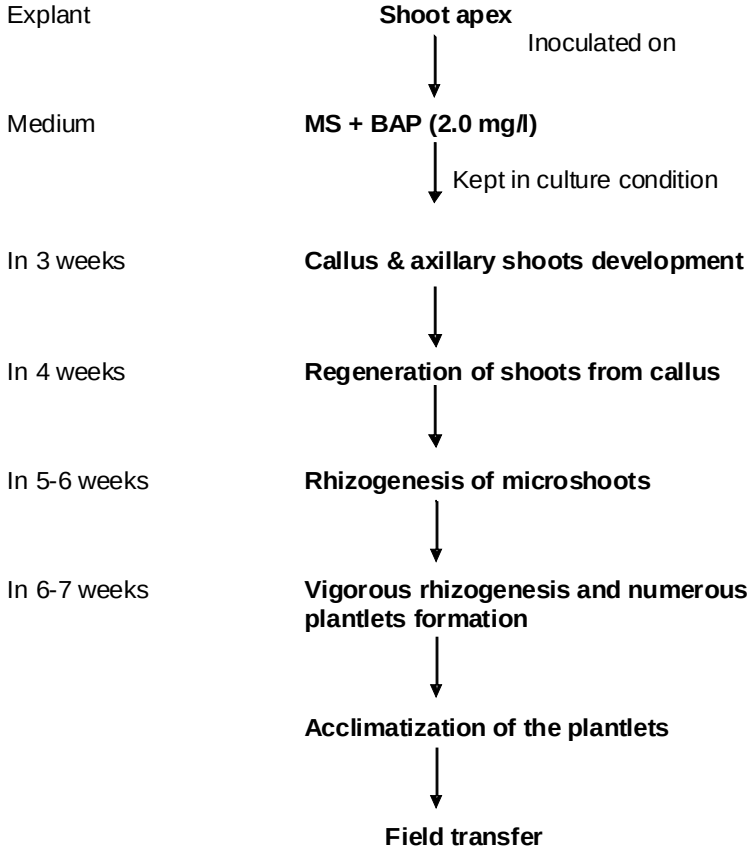
Shoot-apex and nodal explants were selected for the present experiment because actively growing young parts of plants generally show the highest morphogenetic and regenerative capacity (Kukulezanka 1982). Similar to our finding, Cellarova et al. (1984), Venkataraman and Ravishankar (1986), Repcakova et al. (1986) have also reported shoot-apex and nodal explants suitable for micropropagation.

BAP (2.0 mg/l) was found suitable for direct regeneration of plantlets from shoot-apex and also for plantlets differentiation from callus. BAP was found more suitable than KIN (Table 1&2). Similar findings were reported by Cellarova (1992) and Repcakova et al. (1986). Rhizogenesis was achieved on the same medium which was used for shoot development. Efficient single stage multiplication was also reported by Venkataraman and Ravishankar (1986) on 2,4-D and Kinetin medium. However, our report is novel in the sense that we did not utilize auxin for the present experiment.

Similar to our findings, Cellarova (1992) and Repcakova et al. (1986) reported BAP (2.0 mg/l) the most suitable for direct regeneration of plantlets from shoot-apex and also regeneration from callus.

Tissue cultured plants grown under high humidity conditions fail to develop cuticle. Hence, when they are transferred to field conditions in low humidity, they transpire fast. Therefore, to minimize transpiration loss, the plants were covered with polythene film in which vents were made for aeration. Thus, the work provided the protocol for micropropagation of

Micro-propagation protocol :



peppermint;

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ANTAGONISTIC PROPERTIES OF DIFFERENT BACTERIA ISOLATED FROM SALADS

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Abstract : Salads viz. carrot, coriander and cucumber collected from various local markets including producers around Jaipur city showed presence of a number of gram positive as well as gram negative bacteria; however, the population of the later was more. The isolates, based on biochemical tests, tentatively belonged to *Lactobacillus, E. coli, Enterobacter, Pseudomonas, Bacillus* and *Streptococcus*. Out of 110 isolates, some of these showed inhibitory effects on the human pathogens especially on *E. coli, Salmonella typhi* and *Staphylococcus aureus*. The synthesis of antibacterial compound (bacteriocin) from one of the isolate MRS-4 showed highest production at pH of 6.5 and temperature of 37^{0}C.

Key words: Bacteriocin, *E.coli*, Food borne pathogens, *Lactobacilli*, Salads

INTRODUCTION :

Salad is a mixture of fresh vegetables and fruits; eaten raw or partially cooked that promotes good health but, at the same time they harbour a wide range of microbial contaminants. Salads are good source of antioxidants and phytonutrients. They are low in calories and are rich in complex carbohydrates, vitamins and minerals. One health benefit of consuming salad is an increase in fiber intake. Salads provide the body with a lot of fiber which result in lower calorie intake and cholesterol level.

Pathogens on edible plants present a significant potential source of human illness. A significant portion of enteric pathogens can persist on the surface and proliferate. These pathogens can increase the occurrence of food-borne diseases. Fresh vegetables and fruits become contaminated with microorganisms during production, harvest, packaging, and distribution (Bartz and Wei, 2003).

Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable. One such type of incidence occurred in Japan in 1996 in which 11,000 people were affected and about 6,000 pathogenic cultures were confirmed resulted in the death of three children by infection of *Escherichia coli*. The most common bacterial enteropathogens associated with fruits and vegetables are *Salmonella sp., E. coli etc.* (Thunberg *et al.*, 2002; Beuchat, 2002).

The ill-health because of consumption of contaminated fruit juices at several places in India and elsewhere are also reported (Bhaskar *et al.*, 2004; Chumber *et al*., 2007; Ghosh *et al*., 2007). Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *Staphylococcus aureus* (Buchmann *et al*., 1999). In India, the presence of coliforms and *staphylococci* in kinnow and mandarin juices in Patiala city could be reported (Ganguli *et al*., 2004). Similarly, coliforms were observed in fresh fruit and vegetable juices sold by the street vendors of Nagpur city (Titarmare *et al.*, 2009).

Lactic acid bacteria play an important role in the preservation, microbiological stability and production of aroma compound in these products. The preservative effect is mainly due to the acidic conditions that these bacteria create in food during their development but, they are capable of producing and excreting inhibitory substances other than lactic and acetic acid. These include hydrogen peroxide, ethanol, diacetyl, carbon dioxide, bacteriocin or antibiotic-like substances (De Vuyst and Vandamme, 1994a). Most of the bacteriocins from lactic acid bacteria have been isolated from species of the genus *Lactobacillus* because of the diversity of its species and habitats (Klaenhammer, 1988; De Vuyst and Vandamme, 1994b).

Several strains of lactic acid bacteria isolated from commercial salads were active against coliforms, *Enterococci, Aeromonas hydrophila, Listeria monocytogenes, Salmonella typhimurium* and *Staphylococcus aureus*, when tested in solid agar plates, in contaminated salads and juice of vegetable salads.

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The highest activity against many types of pathogens was demonstrated by *Lactobacillus casei* (Vescovo *et al.*, 1996). Application of bacteriocin producing lactic acid bacteria such as *Lactobacillus casei* strains had a remarkable inhibitory effect on the growth of indigenous micro flora and pathogens inoculated in mixed salad vegetables (Vescovo *et al.*, 1996). Lactic acid bacteria inhibit the growth of food-borne pathogens by producing bacteriocin, generating H_2O_2 and producing organic acid and therefore lowering the pH (Breidt and Fleming, 1999; Bennik *et al.*, 1999).

As the salads have a very high consumer preference and eaten raw or partially cooked due to their various important properties; the present study was undertaken to determine the antagonistic properties of some of the bacteria isolated from salads towards human pathogens.

MATERIALS AND METHODS:

Collection of salad samples

Different salads such as carrot, coriander and cucumber were purchased from various local markets (Vaishali Nagar, Jhotwara, Sodala, Achrol and Chandwazi) of Jaipur city and placed in sterile container. The samples were transferred to the laboratory and analyzed within two hours from procurement. Total fifteen samples were collected and processed for the isolation of bacterial colonies.

Isolation, purification and characterization of bacterial isolates

Isolation of different bacterial strains was determined after imposing following main and sub-treatments. Main treatments were (i) washing with ordinary tap water and (ii) washing with warm tap water (40° C). The sub treatments were performed by (i) peeling of the respected samples and (ii) without peeling. 20g of each salad sample was washed with 200 ml ordinary tap water for two minutes. Then rinsed salad sample was divided into two parts equally; one part of salad sample (10g) was used without peeling and the other part was peeled off and transferred in sterilized 250 ml conical with 100 ml of sterile distilled water separately. The sample was finally placed on an orbital shaker at 100 rpm for 10 minutes. The isolation of the colonies was then performed using tenfold serial dilution and then spreaded on Nutrient Agar, MacConkey Agar and MRS Agar media plates and purified by several streaking at 37°C. Similar procedure was used for the isolation with the sample washed with warm tap water (40°C) (Rajvanshi, 2010). The purified isolates of respective medium were characterized on the basis of morphological analysis and biochemical tests.

Determination of production of lactic acid

A loopful of the purified bacterial isolate from MRS Agar plate was aseptically transferred to 20 ml MRS broth (pH 6.5) and incubated for 24 h at 37°C. After the completion of growth, the broth was centrifuge at 10000 rpm for 20 min at 4 °C. The supernatant was collected in sterilized eppendroff tubes and **added drop-wise to Uffelman's reagent, prepared by adding two drops of 1N ferric chloride to 10 ml of 1% phenol solution. The color of solution turns from bluish violet to yellow, indicating the presence of lactic acid (**Walker and Stiles, 2008). The production of lactic acid was also confirmed by paper chromatography using the solvent system acetone, water, chloroform, ethanol and ammonia in the ratio 60:2:6:10:22. The spot developed was visualized by spraying with a solution of bromophenol blue (0.2%) and methyl red (0.2%) in 70% methanol and RF value was calculated (Lee, Heo, 1998).

Antagonistic interaction of isolates against different human pathogens

Three pathogenic cultures *Staphylococcus aureus* MTCC 3160, *Salmonella typhi* MTCC 733 and *Escherichia coli* 901 were used as test organisms for the evaluation of antagonistic properties of the bacteriocin produced from the MRS medium grown cells. The inhibitory activity was determined by using the supernatant of the broth after adjusting its pH to 7.0 by means of 1M NaOH to exclude antimicrobial effect of organic acid. The method adapted for the evaluation of antagonistic property was agar well diffusion assay (Vescovo *et al.*, 1993). The assay involved seeding of Mueller Hinton agar plates with test organisms and introducing 100 ìl of supernatant into the well of a diameter of 6 mm. The plates were incubated at 4°C for 1 h for diffusion of the cell free extract and then incubated at 37°C for 24 h for the development of zone of inhibition.

Development of seed inoculum

The seed inoculum for growth profiling was prepared in MRS broth by inoculating with a loopful of cells and incubated at 37°C for 24 h without agitation. The turbidity of broth was adjusted with sterile MRS and nutrient medium to a turbidity of 0.5 Mac Farland standards, which resulted in a suspension containing approximately 1.5×10^8 bacteria/ml.

Growth profiling of lactic acid bacteria

The growth of lactobacilli screened on MRS medium was investigated by inoculating 1% (v/v) of seed inoculum in sterile MRS broth with the initial pH of 6.5 and incubated for 18 h, 24 h, 48 h and 72 h at 37°C in an anaerobic condition.

RESULTS AND DISCUSSION

Based on our preliminary investigation about the abundance of different bacterial isolates and the antimicrobial activity of some of the bacteria isolated from salads, showed that maximum population belong to the genus *Bacilli* (79%) followed by *Cocci* (21%) as shown in Figure 1. The presence of *Lactobacilli* was reported from carrot while *Leuconostoc* was screened from cucumber and coriander as given in Table 1. Similar type of work has been performed by Uhlman et al., 1992 and Vaughan *et al*., 1994. They isolated antimicrobial substances producing vegetable associated lactic acid bacteria.

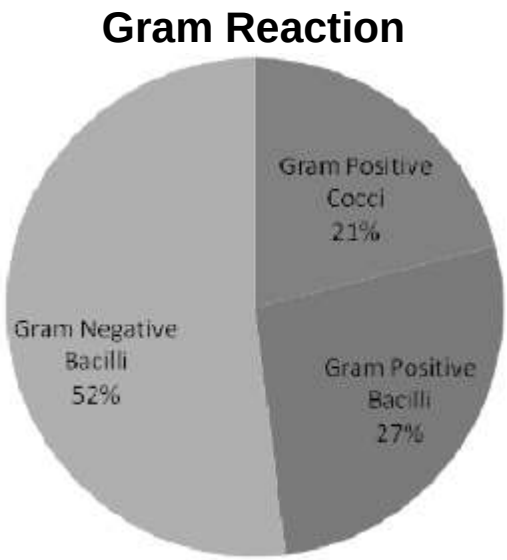


Figure 1: Abundance of different bacteria isolated from salads.

Table 1: Identification of isolates on the basis of biochemical tests.

Name of bacteria	No. of bacteria (cfu× 10^4/ ml after washing)		
	Carrot	Coriander	Cucumber
E.coli	04	05	04
Enterobacter	03	04	03
Staphylococcus	02	06	04
Streptococcus	03	02	02
Pseudomonas	05	04	04
Klebsiella	02	03	01
Citrobacter	01	01	02
Bacillus	08	13	06
Lactobacillus	02	-	-
Lactococcus	-	01	01
Not identified	06	05	03

Studies on antagonistic properties of different isolates: Out of 110 strains, thirteen isolates were evaluated for their antagonistic properties using *E. coli* MTCC 901 as pathogen and the results of zone of inhibition obtained are represented in Table 2. The observation of the table revealed that all the isolates inhibited *E. coli* confirming the presence of antibacterial compounds in the broth with maximum inhibition by *Pseudomonas.* It was also noted from the table that all the MRS grown cells also inhibited the pathogen with maximum inhibition by MRS-4. These isolates are lactic acid producing bacteria while Co10k is a non lactic acid producing bacteria. The present study confirms the findings of Klaenhammer, 1988; De Vuyst and Vandamm, 1994a,b; Vescovo *et al*., 1996; Breidt and Fleming, 1999; Bennik *et al.*, 1999. They isolated bacteriocin producing lactic acid bacteria from juices, fruits and vegetable salads. Similarly, epiphytic species of *Pseudomonas* have been identified and commercialized for the control of postharvest decays caused by fungi and bacteria in fruits (Janisiewicz, *et al.*, 2002).

Table 2: Antagonistic isolates against *E. coli* with inhibition zone.

Salads	Isolate No.	Diameter of inhibition zone (mm)	Genus of isolates
Carrot	Ca1c	12 ± 2.828	*Bacillus*
	Ca2c	10 ± 1.414	*Bacillus*
	Ca4c	12 ± 3.423	*Streptococcus*
	MRS1	10 ± 2.828	*Lactobacillus*
	MRS2	8.5 ± 0.707	*Lactobacillus*
Coriander	Co8g	10 ± 1.414	*Bacillus*
	Co10k	15 ± 3.423	*Pseudomonas*
	MRS3	10 ± 2.828	*Lactococcus*
Cucumber	Cu11e	12 ± 1.414	*Bacillus*
	Cu12c	10 ± 1.414	*Streptococcus*
	Cu12e	12 ± 2.828	*Bacillus*
	Cu13h	12 ± 3.423	*Streptococcus*
	MRS 4	12 ± 1.414	*Lactococcus*

*Values are mean ± SD and significant at ($p < 0.05$)

Based on the observation from table 2, we have selected only three isolates (MRS-1, MRS-4 and Co10k) for further studies. Figure 2 shows the antagonistic properties of these isolates against three

Table 3: Effect of treatment on *E. coli* 901 as monitored by absorbance at 600 nm.

Time (h)	Supernatant (ml)	*E.coli* Control	*E. coli* + MRS-1	*E. coli* + MRS-4	*E. coli* + Co 10k
24	0.5		0.26±0.020	0.24±0.042	0.21±0.028
	1.0	0.32±0.010	0.23±0.010	0.21±0.048	0.18±0.042
	2.0		0.18±0.020	0.16±0.057	0.14±0.057
48	0.5		0.25±0.028	0.21±0.057	0.19±0.048
	1.0	0.41±0.010	0.19±0.014	0.16±0.052	0.14±0.028
	2.0		0.13±0.016	0.11±0.047	0.10±0.057

*Values are mean ± SD and significant at ($p < 0.05$).

human pathogenic organisms as mentioned in the material and methods section. We have also carried out the different treatments on *E. coli* using the supernatants of three antibacterial synthesizing isolates as shown below.

Treatment 1) 9 ml NA broth + 1 ml *E. coli* seed inoculums (as control)

2) 8.5 ml NA + 1 ml seed + 0.5 ml supernatant

3) 8.0 ml NA + 1 ml seed + 1.0 ml supernatant

4) 7.0 ml NA + 1 ml seed + 2.0 ml supernatant

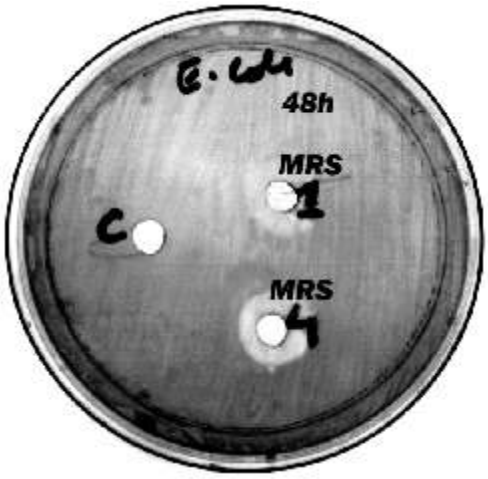
The result of this experiment as observed in the decrease in cell O. D. is represented in Table 3.

It was found that there was a drastic decline in absorbance indicating the cell lyses of *E. coli* which was due to the antibacterial activity of bioactive compound synthesized by lactic acid as well as non lactic acid bacterial isolates screened from salads. To confirm the decrease in cell optical density of *E. coli*, we have performed the viable cell count as given in Table 4 and found the reduction in cell number. Similar type of work have been performed by Yazid *et al.*, 1999, they checked antibacterial activity of four lactic acid producing *Bifidobacterium* strains against food borne pathogens by study cell optical density and viable count of *E.coli* and other used pathogens.

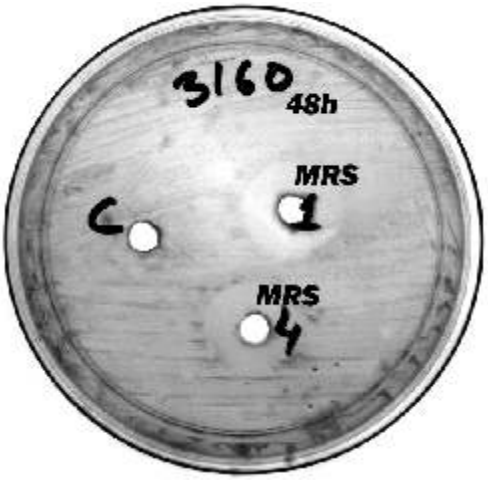
Table 4: Viable cell count (CFU×10^6/ml broth) of treated *E. coli* broth.

Time (h)	Supernatant (ml)	*E. coli* Control	*E. coli* + MRS-1	*E. coli* + MRS-4	*E. coli* + Co 10k
24	0.5 ml		182±8.265	164±5.637	136±8.465
	1.0 ml	304± 8.485	114±6.845	104±8.385	84±5.867
	2.0 ml		72±5.657	56±5.462	32±2.426
48	0.5 ml		48±7.271	40±5.245	24±5.457
	1.0 ml	415±7.071	28±8.645	24±5.657	16±2.628
	2.0 ml		12±2.828	06±1.414	03±2.121

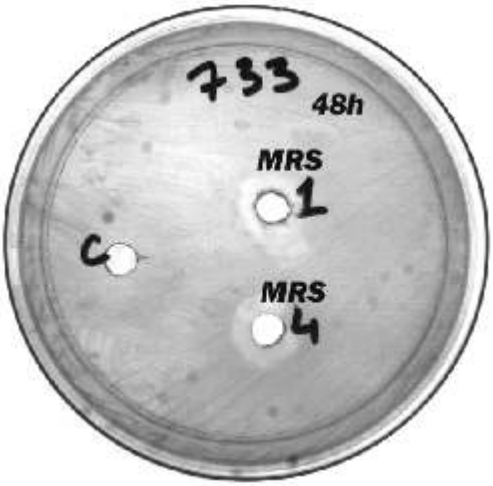
*Values are mean ± SD and significant at ($p < 0.05$).



MRS1 and MRS4 against *E. coli* 901



MRS1 and MRS4 against *S. aureus* 3160



MRS1 and MRS4 against *S. typhi* 733

Co10k against *E. coli* 901

Co10k against *S. aureus* 3160

Co10k against *S. typhi* 733

Figure 2: Antagonistic activity of supernatant against all human pathogenic bacteria.

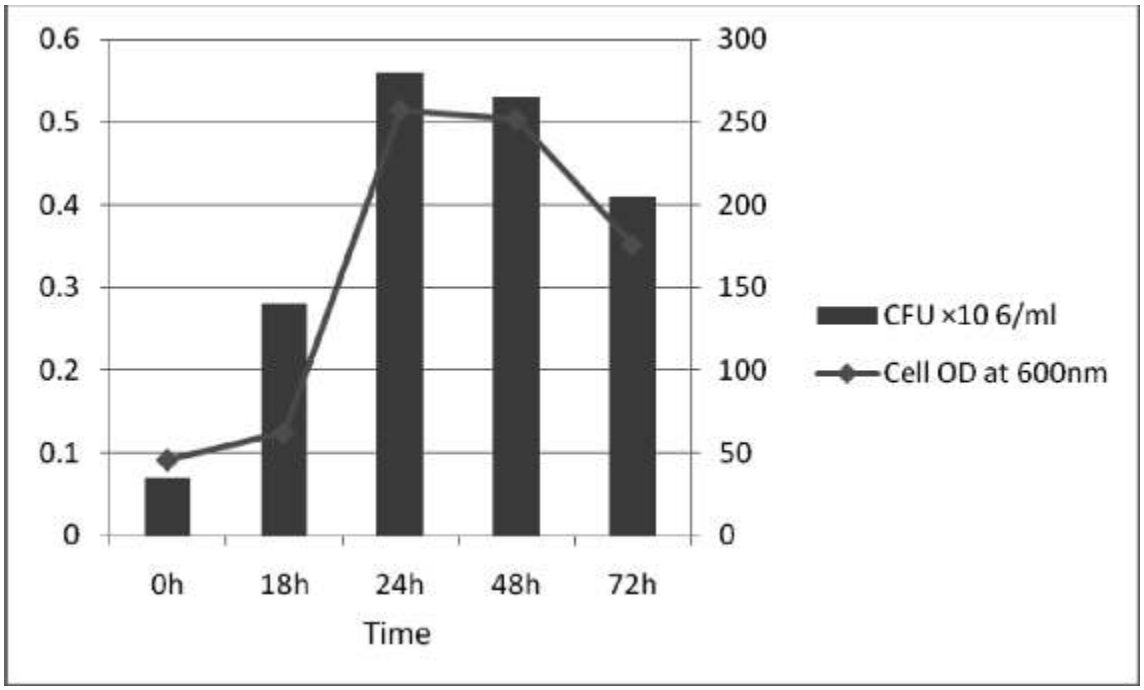


Figure 3: Growth profiling of MRS-4 in terms of O.D. as well as CFU.

Growth kinetics and production of bacteriocin from MRS-4:

The growth profiling of MRS-4 (*Lactococcus*) was investigated in the liquid broth at optimal conditions. Different flasks were used for different time intervals to analyze the increase in cell optical density as well as colony forming unit. The result of increase in cell density with respect to time is shown in Figure 3.

It indicates that maximum cell biomass was achieved during 24 h of incubation and remained more or less constant till 48 h of incubation as shown by the number of colonies observed on the agar plate. As far as the production of bioactive compound is concerned it starts from 18 h of incubation and remains constantly produced throughout the stationary phase. The zone of inhibition as recorded against pathogens is represented in Table 5. Similar observations were reported by earlier investigators while studying growth profiling of Lactic acid bacteria (Leroy and De Vuyst, 2001; Todorov and Dicks, 2005) by studying growth profiling of Lactic acid bacteria.

Table 5: Production of bacteriocin from MRS-4 at different time intervals, represented by zone of inhibition (mm).

Time (h)	*S. aureus* 3160	*E. coli* 901	*S. typhi* 733
18	10±1.414	9.5±0.707	9.5±0.707
24	14±1.414	12±1.414	12±1.414
48	14±2.828	12±2.828	12±2.828
72	14±1.414	12±1.414	12.5±0.707

*Values are mean ± SD and significant at ($p < 0.05$).

CONCLUSION

Based on our preliminary work, it can be concluded that the metabolite produced by MRS-4 could be used as biocontrol agent for inhibiting the human pathogenic organisms. The bacteriocin produced from this isolate has potential application as a bio preservative in the food industry.

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Survival, Growth and Production of *Penaeus monodon* in Modified- Extensive and Semi Intensive Culture Systems of Andhra Pradesh, India

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Abstract : An analysis was made on the stocking density, survival, growth and production of *Penaeus monodon* in semi intensive (SI) and modified extensive (ME) shrimp culture ponds located in East Godavari district of Coastal Andhra Pradesh, India. Survival of shrimp in the ME systems with low stocking densities is higher (56 - 88%) than in the SI systems (12.3 – 52%) with higher stocking densities. Growth of shrimp is dependent not only on the stocking density but also on the management practices. Although the percent survival is more in ME system the growth is much faster in SI system owing to better management practices. Weight gain of shrimp was more rapid in the SI system than in the ME system initially up to 90 days of culture (DOC) but the trend was reverse in the later age of culture period. The optimum stocking density for achieving maximum production in the area is suggested as 10-15 individuals / m^2 in SI ponds and 3-5 individuals / m^2 in ME ponds.

Key words: Shrimp, Stocking density, weight gain, Biomass production

INTRODUCTION :

Penaeus monodon is a fast growing euryhaline and eurythermal shrimp, cultured on a large scale in many Asian countries including India. Production of the shrimp in the culture system depends on a wide range of factors, the most important being the stocking density, which influences the growth and survival rates of the stock (Allan and Maguire 1992, Martin *et al.*, 1998, Ahmed et al., 2000 and Soundarapandian & Gunalan, 2008). An inverse relationship is known to exist between the stocking density and the survival and growth rates (Sandifer & Smith, 1976, Emmerson & Andrews 1981, Ahmed *et al.*, 2000). The susceptibility of shrimp to diseases increase with increase in stocking density (Hanson and Goodwin 1977, Baticados *et al.*, 1986, Doubrovsky *et al*., 1988) and the increase of density also increases the pressure on natural food resources (Hopkins *et al.*, 1988). Although low stocking densities tend to be risk free and are economically not viable. On the other hand, stocking the ponds beyond their carrying capacity is risky and may lead to total failure. A detailed analysis of the relationship between the stocking densities and the survival rates, growth and production of shrimps in culture ponds in a defined locality would prove especially useful in determining the optimum stocking densities to be adopted in the culture ponds as relevant to the prevailing conditions in the locality.

In India, where brackish water shrimp culture has expanded as a major industry, spread over vast area (85,000 ha), three types of culture activities are in operation, designated based on their stocking densities, as extensive, modified extensive and semi intensive types.

An investigation has, therefore, been undertaken to compare the survival rates, growth and production of *P. monodon* in semi intensive (SI) and modified extensive (ME) types of culture ponds located in East Godavari district of Andhra Pradesh (India).

Materials and Methods

Culture ponds

Eight culture ponds, four of them belonging to SI type and the rest to ME type, located in Chollangi village (East Godavari district) of Andhra Pradesh, India were selected. For convenience the selected ponds were designated as SI A, SI B, SI C, SI D (Semi–Intensive) and ME1, ME2, ME3 and ME4 (Modified - Extensive). The physico-chemical characters of the selected ponds were broadly similar except for minor differences. The rate of water exchange in ME ponds was 13-16 % per week, whereas it was 19-26 % per week in SI ponds. The feed conversion ratio (FCR) also varied from 1.3 to 2.9 in ME ponds and 1.96 to 3.8 in SI ponds. The quantities of inputs used were much higher in SI ponds as compared to ME ponds (Tables 1 & 2). All the ponds were stocked with post larvae (PL 20 stage) produced in hatcheries.

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TABLE 1 : ***Stocking densities, water exchange rates, fertilization, FCR, survival and growth in SI ponds at Chollangi***

	Ponds			
	SI A	SI B	SI C	SI D
Area (ha)	1	0.55	0.6	1
Initial Stocking density/m^2	15	20	20	15
Survival (%)	46.5	12.3	25.6	42
Biomass Kg/(ha)	2575	772	1583	2225
Feed used (Kg)	5055	1636	2183	5505
Feed conversion ratio (FCR)	1.96	3.8	2.29	2.39
Shrimp growth/ week/ g	1.98	2.4	2.03	1.55
Biomass (Kg/ha/day)	17.4	6.44	13.2	15.6
Water exchange (%)	19	26	20	24
Culture period (days)	148	120	120	142
Length of prawn at harvest (Cm)	16.5	15.2	15.0	15.4
Weight of shrimp at harvest (g)	38	32	29.5	20
Cao (Kg)	3000	450	240	3300
Lime (Kg)	1520	920	800	720
Urea (Kg)	64	42	56	28
Di Ammonium Phosphate (DAP) (Kg)	16.5	12	10	15
Dolomite (Kg)	1000	410	510	1230
Zeolite (Kg)	125	75	75	75
$KMnO_4$ (Kg)	-	-	-	10
Vitamin C (Kg)	0.3	0.08	0.09	0.38
Malachite green (Kg)	0.2	-	-	1.2
Probiotics (Kg)	43.5	15	12	50

Sampling

Sampling commenced from 30th day of post-stocking in ponds, and continued at fortnightly intervals till the day of harvesting. At each sampling time, data was collected on the survival, growth rates and production of shrimp in each pond. Data has been collected for a single crop which fell between February to May 2008.

Survival rates

Random sampling method employing cast net operation in the pond was used for the estimation of percent survival rates, using the formula.

Percent survival (%) =

$$\frac{\text{Actual number of shrimps caught/m}^2}{\text{Expected number of shrimp/m}^2} \times 100$$

Growth rate and Biomass production

Growth rate was estimated from the average body weight of shrimp obtained during each sampling time. Biomass Production was estimated at different days of culture period by multiplying the % survival with the average weight of shrimp.

TABLE 2 : Stocking densities, water exchange rates, fertilization, FCR, survival and growth in modified extensive (ME) ponds at Mulakuddu

	Ponds			
	ME 1	ME 2	ME 3	ME 4
Area (ha)	1.3	0.8	1.0	0.4
Biomass Kg/(ha)	500	1125	912	687
Stocking density (Individuals/m^2)	3	3.5	5.4	3.75
Biomass (Kg/ha/day)	3.7	8.85	6.37	4.6
Water exchange (%)/week	13	16	15	14
Culture period (days)	135	125	143	150
Survival (%)	56	88	71	43
Feed used (Kg)	887	1200	1370	816
Feed conversion ratio (FCR)	1.36	1.3	1.5	2.9
Shrimp growth / week / g)	2.349	2.04	1.98	1.91
Length of shrimp at harvest (cm)	14.8	14.8	15.8	18.0
Average weight of Shrimp at harvest (g)	29.0	29.1	36.4	42.0
Urea (Kg)	20	25	35	11
Superphosphate (Kg)	25	20	25	15
Dolomite (Kg)	290	125	150	150
Lime (Kg)	150	150	200	100
Zeolite (Kg)	50	-	-	2.5
Planktamin (Kg)(Mineral supplement to enhance growth of phytoplankton)	1	1	1	1

Feed conversion ratio

It is the ratio between the feed intake and weight gain. It was estimated on the basis of total feed used during the culture period and the corresponding production at the time of harvest.

Feed conversion ratio = Total Feed used / Weight gain

Statistical analysis

ANOVA and student's t- test were employed to determine the significance of difference in the growth rates of shrimp within and between farms. The extent of relationship between stocking densities and survival rates were determined by applying Karl Pearson's correlation coefficient (r). The significance level was set at P = 0.05.

Results

The stocking densities in SI ponds ranged from 15 to 20 / m^2 while those of ME ponds were within the range of 3- 5 /m^2. Details of the characteristic features of the selected ponds are furnished in Tables 1 & 2. The survival, growth and production varied markedly between SI and ME ponds ($P<0.05$).

***Survival* rates**

Semi-intensive ponds (SI): The percent survival rates were low ranging from 12.3 % (SI B) to 52% (SI D) at 120 days of culture (Fig.1). There was a gradual decline of survival rate in all the ponds, whereas in SI B, a rapid decline in survival rate was observed from 45th day onwards due to low dissolved oxygen level. Differences in the survival rates of shrimp in the four SI ponds at different times of culture are significant

(P<0.05). Further, a negative correlation was observed between stocking density and survival rates (r = - 0.9).

Modified extensive ponds (ME) (Fig. 2)*:* Survival rates of shrimp in all ponds were high, ranging from 56 to 88% at 120 days of culture. The percent survival remained almost uniform throughout the culture period in ME 2 pond where as gradual decline was noted in the other ponds. Differences observed in the survival rates of shrimp in the four ponds at different ages are significant (P <0.05).

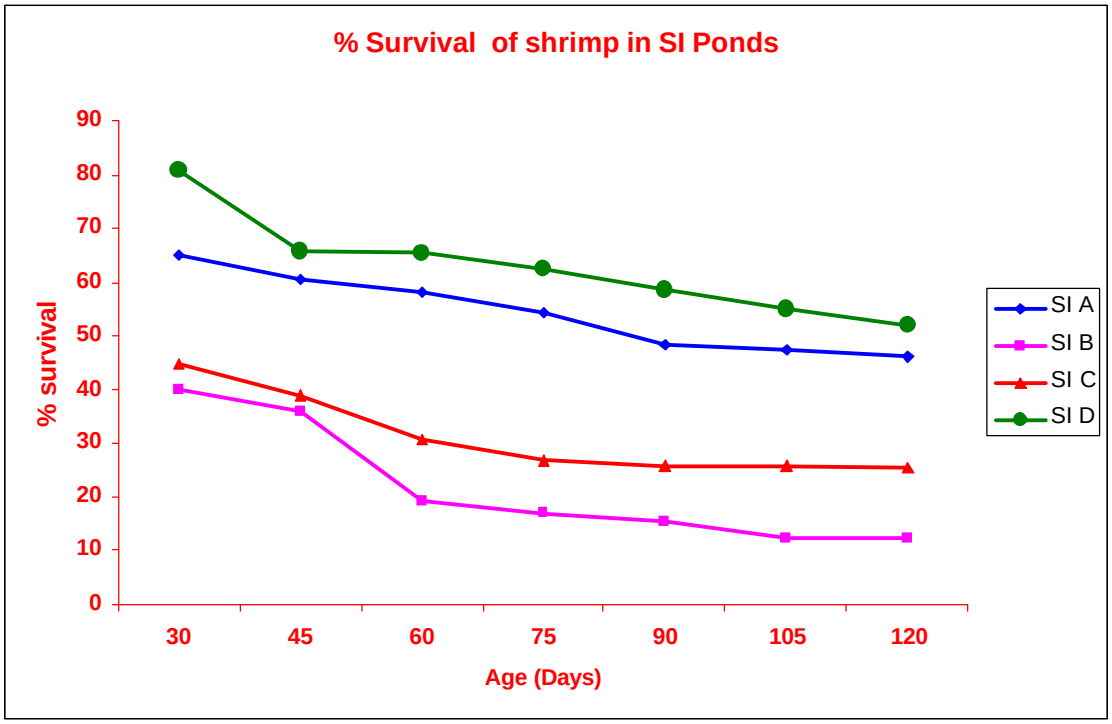

Fig. 1 : Survival rate of shrimp in Semi - Intensive ponds

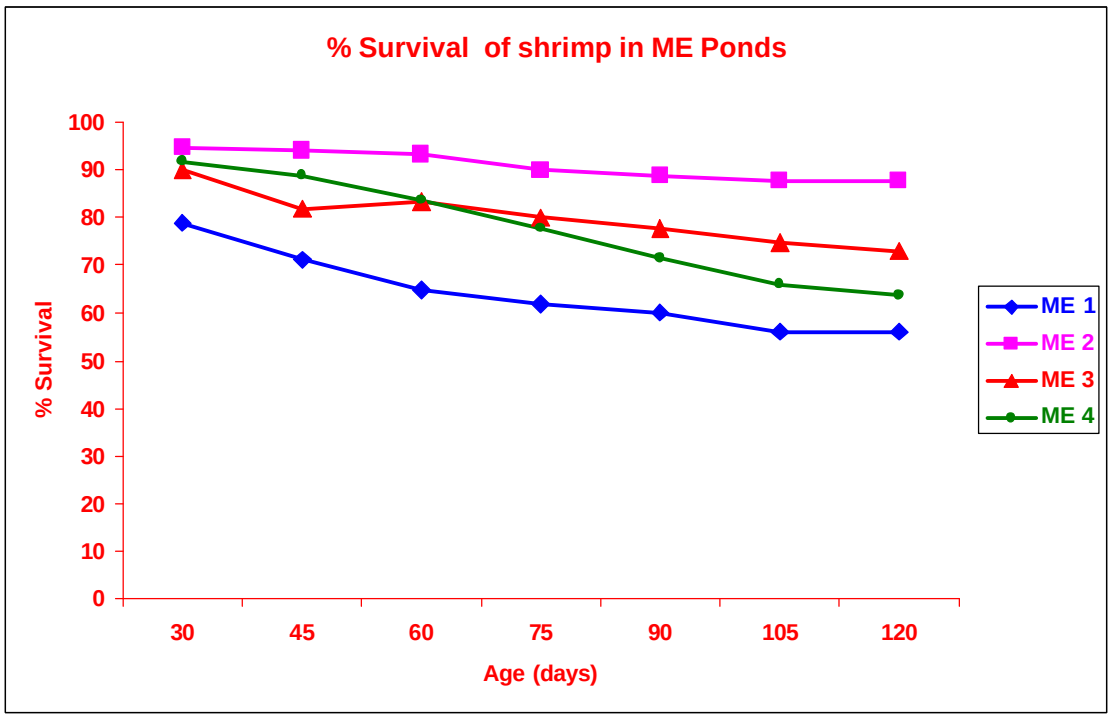

Fig. 2 : Survival rate of shrimp in Modified-Extensive ponds

Growth *rates*

In all the eight ponds, growth rate was assessed by average weight of shrimp, which showed a linear relationship with age giving a positive correlation (r = 0.99 to 1.0). Whereas, a negative correlation was observed between the weight of the shrimp and % survival (r = - 0.9). Initially, the growth rate was similar in all ponds up to 45 days of culture, except in ponds ME 2 and ME 3 where the % survival was maximum. ANOVA of data resulted significant differences in the weights of shrimp in both SI and ME ponds ($P < 0.05$).

SI ponds (Fig. 3)*:* The growth rates varied

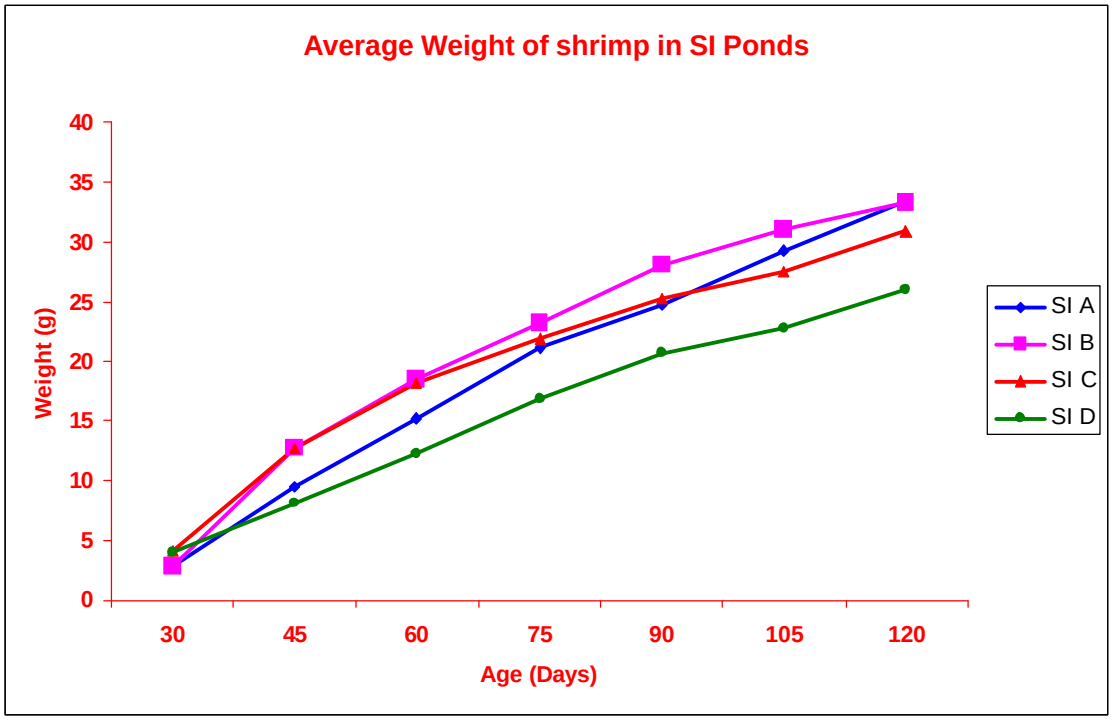

Fig. 3: Growth rate of shrimp in Semi-Intensive ponds

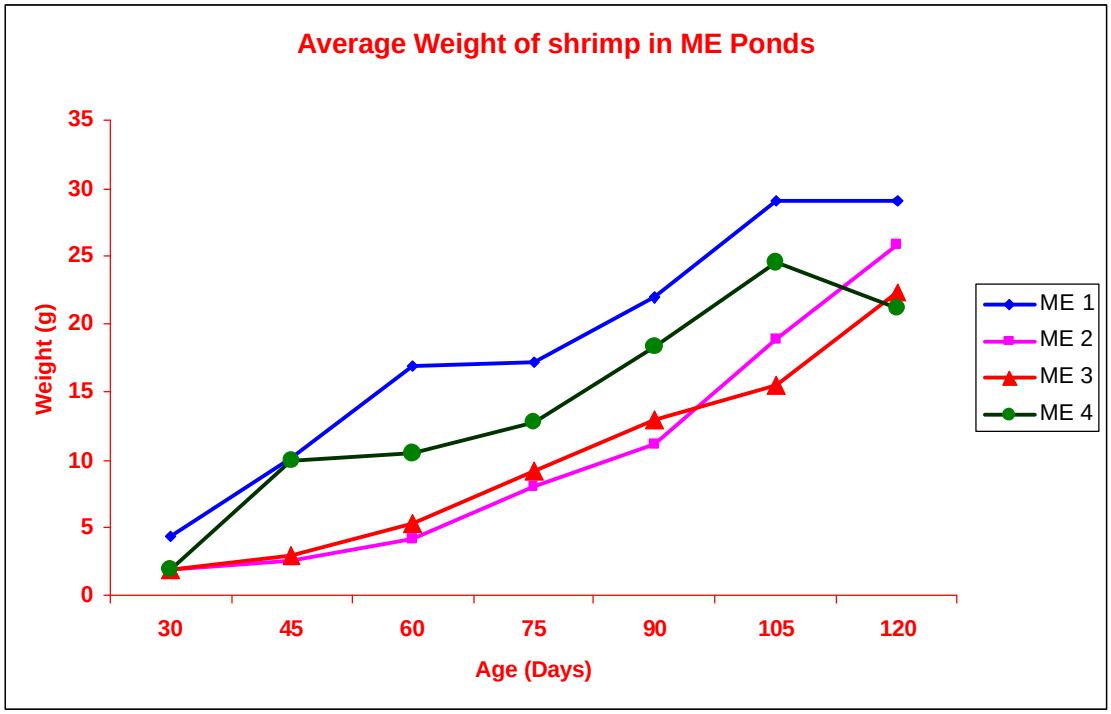

Fig. 4: Growth rate of shrimp in Modified - Extensive ponds

significantly between ponds at all ages. Paired comparison of weights taken from sample of shrimp from the all the four ponds at 120 days of culture, revealed that the average weight of shrimp from pond SI D to be significantly lower than that of shrimp from other ponds. Data subjected to ANOVA revealed that there was significant difference in the weights of shrimp between and within ponds.

ME ponds (Fig. 4): The growth rates varied considerably at 120 days of culture. Comparison of weights of shrimp from the four ponds at the age of 120 days revealed that the average weight of shrimp in pond ME 4 to be significantly lower than that of shrimp in other ponds.

Weight gain

SI ponds (Fig. 5)*:* The weight gain of shrimp in SI B was higher initially before the occurrence of dissolved oxygen problem (45 days of culture), but it fell down since then and showed influence throughout the culture period. Data subjected to ANOVA revealed the significant difference in the weight gain of shrimp between ponds $P < 0.05$.

ME ponds (Fig. 6)*:* The weight gain of shrimp is minimum in case of ME 4 pond (6.6 g) at 120 days of culture. ANOVA revealed that there was no significant difference in the weight gain of shrimp between and within ponds ($P > 0.05$).

Biomass Production

In SI ponds, the production varied form 772 – 2575 kg/ha and the daily production varied from 6.44 kg (SI B) to 17.4 kg/ha (SI A: Table 1). Production in ME ponds varied from 500-1125 kg/ha. The daily increase in biomass was highest in ME 2 (8.85kg) and lowest in ME1 (3.7 kg) and those of ME 3 and ME 4 were 6.37 kg and 4.6 kg respectively.

Discussion

Stocking density is one of the major limiting factors which influence the growth and survival rates of shrimp in a culture system. Very few studies were ,however, devoted to analyze this relationship in the culture systems with respect to the production. Foster & Beard (1974) and Willis *et al*. (1976) observed the survival and growth of some species of juvenile prawns to be better at lower stocking densities. Sandifer & Smith (1976), Emmerson & Andrews (1981) and Ahmed *et al*. (2000) also found that survival and growth varied inversely with population density. A similar trend was also noted during the present study with more growth in low stocking densities. The survival rate being very high in the ME ponds under conditions of

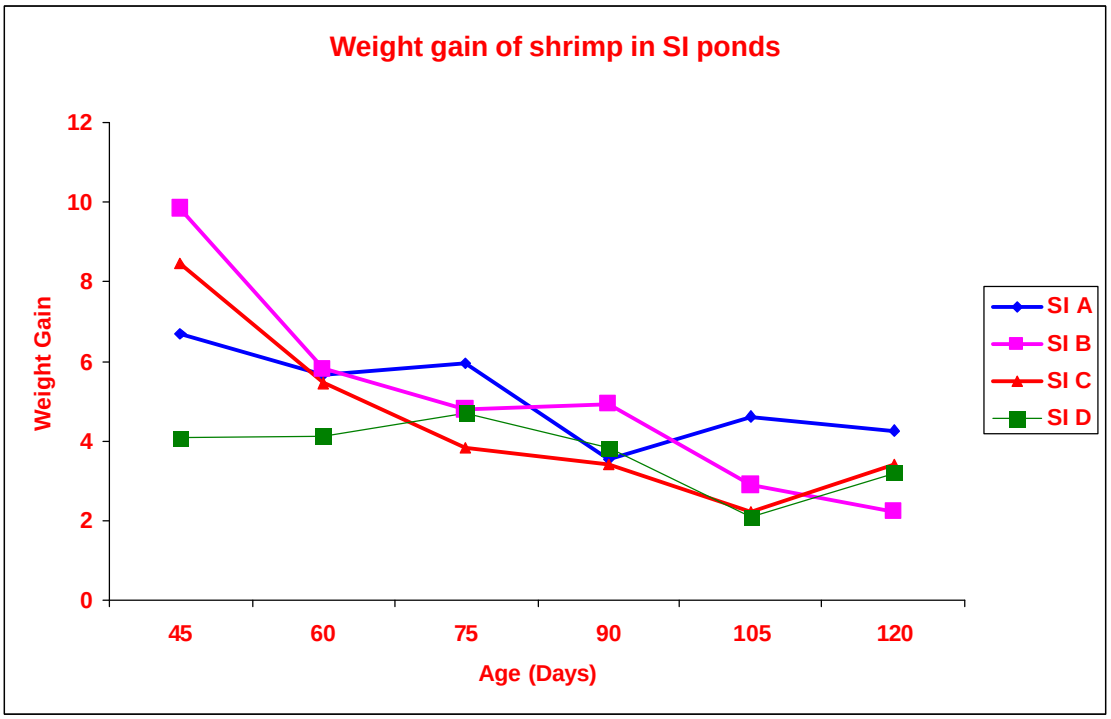

Fig. 5: Weight gain of shrimp in Semi-Intensive ponds

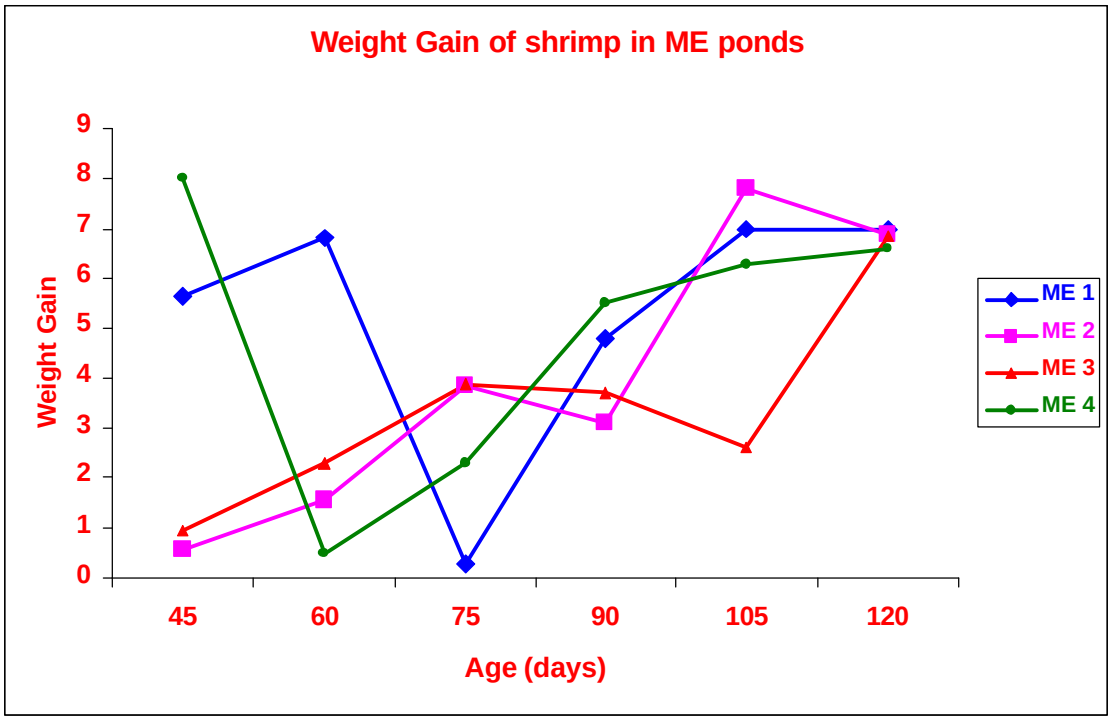

Fig. 6: Weight gain of shrimp in Modified - Extensive ponds

low stocking density as compared to low survival with higher stocking densities in SI ponds. In general, the survival rates recorded during the present study were higher than those reported by earlier workers from other localities in India and other countries (Chakraborty *et al.*, 1985, 1997., Eldani & Primavera,1981 and Ahmed *et al.*, 2000). This seems to be a reflection of the progress achieved in the management practices adopted in culture systems. During the present study, a clear relationship was noted between stocking density and survival rates. Among the four SI ponds, two ponds with lower densities showed fairly high survival rates of 46 % to 52%. Apart from the stocking density, the survival rates apparently depend on other factors including the quality of seed, the pond environmental quality, the quality and quantity of feed used, the rate of water exchange and other management practices.

The growth is dependent on large quantities of fertilizer inputs, abundant natural feed and also on the supplementary feed besides management practices. Growth rate of shrimp depends mainly on the stocking density, the feed utilization and the availability of natural food (Tidwell *et al.*, 2004). Subrahmanyam (1973) has observed growth of *P. monodon* affected by low temperature under laboratory conditions. Verghese *et al*. (1975) observed rapid growth of *P. monodon* coinciding with increase of temperature. During the present study, it was found that slight temperature fluctuations in the course of production cycle were not significant enough to have any role on pond production. Karplus *et al.* (2000) recommended that a grow out period of minimum 140 – 150 days for shrimp to achieve maximum production.

The role of salinity in affecting growth has been suggested by several authors. Subrahmanyam (1973), Verghese *et al.* (1975) and Liao (1977) have observed the direct influence of salinity on growth of *P. monodon.* Chakraborty *et al.* (1986) have observed that the growth was stunted during the period when the salinity was low. In the present study, in both the farms the growth of shrimp was arrested at 30-45 days when the stock had to pass through lower salinities caused by heavy monsoon.

An inverse relationship between stocking density and growth rate was observed by Wyban *et al.* (1987). Total daily feed according to the body weight must be given to the shrimp but the frequency of feeding has no effect on either the growth rate or survival of shrimp (Smith *et al*., 2002). It is found that the growth rates of shrimp in SI ponds is more but the weight gain was high in ME ponds owing to the lower stocking densities.

An analysis of growth rates reported for the various species of shrimp revealed wide variations. Trimble (1980) reported that *P. vannamei* grew 1.28 g

/week at a stocking density of 2.5 shrimp/m^2. Wyban *et al.* (1987) found growth rate to be 1.72 g / week at a stocking density of 5 shrimp/m^2. Liao (1977) and Sundararajan *et al.* (1979) observed faster growth rates have been recorded for *P. monodon* (0.32 to 0.39/g/day).

In our study, the growth rate of 1.9 – 2.3 g/shrimp/week was recorded in the ME ponds with densities of 3-4 shrimp/m^2. In SI ponds, with stocking densities of 15-20/ m^2 almost similar growth rates (1.6 – 2.4) were obtained through judicious use of feed and fertilizers. It is observed that the outbreak of disease in the SI B pond could be due to high stocking density well supported by earlier researchers (Hanson & Goodwin 1977, Baticados *et al*., 1986 and Doubrovsky *et al*., 1988). Martin *et al.* (1998) suggested that the stocking density between 10-20 pls / m^2 is ideal for successful shrimp culture. In the present study, the survival and production was poor in the ponds stocked with more than 20 shrimp /m^2. In the ME ponds stocked with 3 – 5 shrimp/m^2, the survival and growth rates were higher but production was low (500 – 1125 kg/ha). Finally, our study revealed that the maximum production was obtained in ponds with low stocking densities and high survival rates. Based on our study, it is recommended that the ideal stocking density in SI and ME systems to be 10-15 and 3-5 individuals/m^2 respectively.

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Biodiversity of Zooplankton and Zoobenthos at Thane Creek, Mumbai in West Coast of India

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Abstract : The study of Zooplankton and Zoobenthos at 15 stations of Thane Creek during pre-monsoon period (May, 2008) were carried out to assess the water quality and bio-ecoaesthetic value of the creek. Among the major taxonomic groups, Copepoda (8 sp.) was the most dominant Zooplankton. Based on study of Zooplankton, it appeared that the area is mostly preferred by Copepoda, Nauplius spp., *Acardia spinatafollowed by Oithona sp., Miracia efferata and Ectinosoma dentatum etc.* Meiobenthos was dominated by Foraminifera (15 sp.) followed by Ostracoda (10 sp), Polychaetes (10 sp.) and Nematoda (2 sp.), while Macrobenthos was mostly dominated by Gastropoda (15 sp.), Bivalves, Polychaeta and Podocopida (3 sp.) each. An average counts for zooplanktons, Macrobenthos and Meiobenthos were computed as 6055/m^3, 20355/m^2 and 44000/m^2 respectively. Higher diversity indices near the open sea and lower density at effluent discharge point was recorded indicating medium impact of pollution in the study area during low tide.

Key words : Water quality, Pollution, Fauna, Diversity index, Estuary

Introduction

The zooplankton and zoobenthos constitute important links in aquatic food chain. They are good indicators of water pollution. They play a major role in pollution monitoring studies. Zooplankton and Zoobenthos are the basic aspects for environmental impact assessment studies. Due to urbanization there is tremendous pollution pressure on the water of the creek. The present investigation was carried out to focus attention on biotic faunal features of Thane Creek. This creek is located between longitudes 72° 55' to 73° 00'E and latitudes 19° 00' to 19° 15' N to the south of Mumbai harbor **(Fig. 1).** The creek is tide dominated and subjected to the load of industrial effluent and waste dumping sites. The creek joins to Ulhas River estuary which extends east to west meeting the Arabian Sea near Vashi. Studies were carried out on zooplankton and benthos to examine the water quality of the creek. The sampling was done during high tide (HT) and low tide (LT) in summer season (May-2008) in the study area. The sampling locations were identified and selected as industrial effluent (9 LT/HT, 14 LT/HT) and non-effluent discharge points. The objective of the study is to estimate diversity index of zooplankton and zoobenthos in the study region of the Thane creek.

Materials and Methods

The sampling programme covered a distance of about 10 kms between mouths of Arabian Sea towards the point where Thane Creek joins Ulhas River estuary. Total 15 sampling locations were selected covering pollution impacted region of the creek. Sampling programme was conducted for the period of 15 days. Samples were collected both for low tide (LT) and high tides (HT). The water samples for zooplankton analysis were collected by filtering 200 litres of sea water through "Nylobolt Plankton Net # 20 and preserved in 4% buffered formalin immediately. The organisms were identified with the help of available reference (Kameswara Rao et al., 1987; Krishna, 1986; Kasturirangan, 1963 and Wickstead, 1965).

For zoobenthos, bottom sediment sample was collected by Van Veen grab sampler having the area of 0.03 m^2 with the depth of 15 cm. The sediment samples thus collected was sieved through 500µ mesh sieve for Macrobenthos. Core sampler was used to collect the sediment for Meiofauna and sieved through 45µ mesh sieve. The organisms retained on both the sieves were preserved immediately with 4% buffered formalin. Temperature, pH and dissolved oxygen (DO) were analyzed at the site whereas other physico-chemical parameters were analysed in the laboratory. All the analyses were carried out according to Standard Methods (APHA, 1992).

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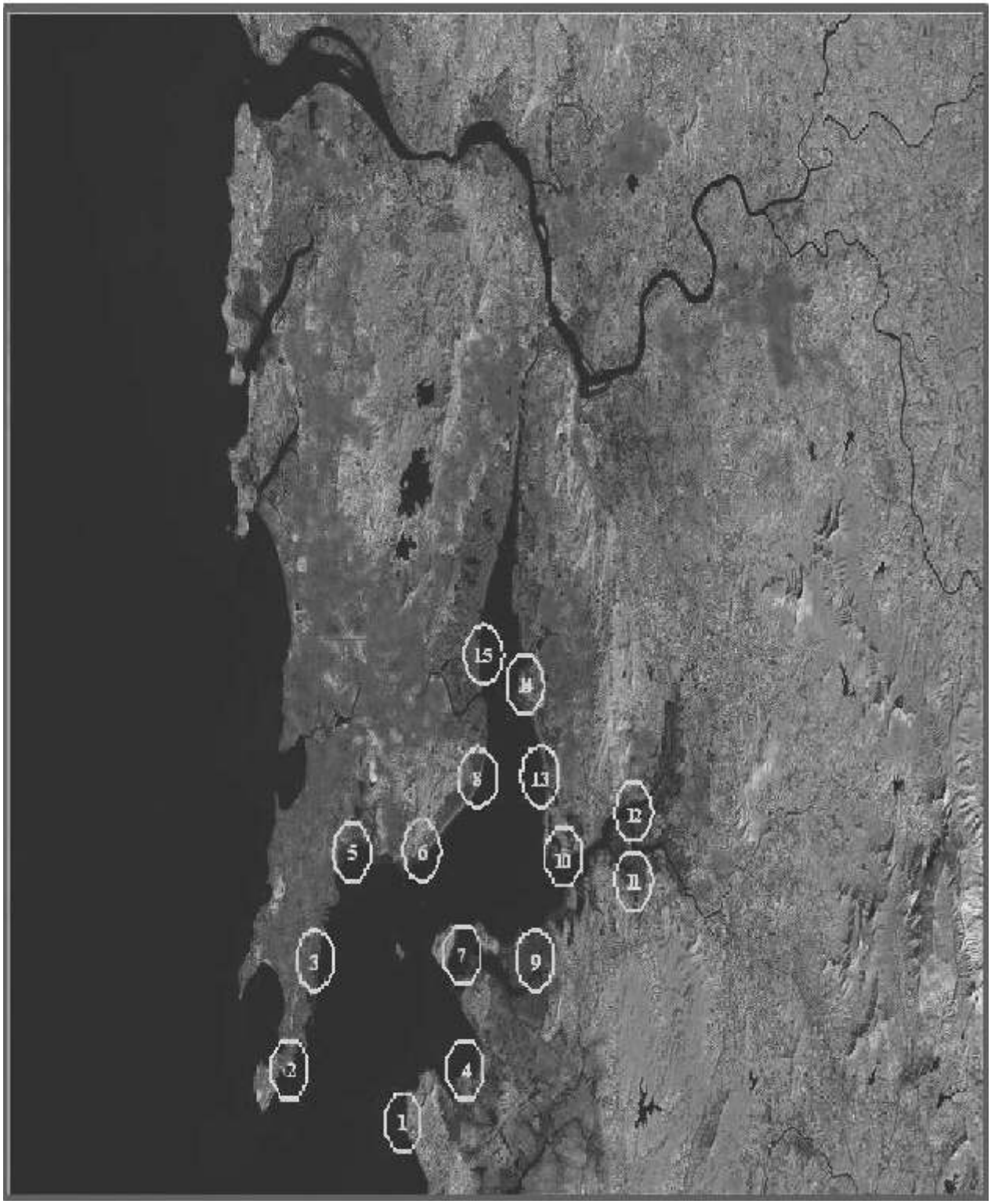

Fig. 1: Sampling locations at Thane Creek, Mumbai

Result and Discussion

The physico-chemical characteristics of sea water in the study area are presented in **Table 1.** The analysis showed that the DO ranged from 2.0 - 4.2 mg/l with a mean value of 3.1 mg/l. BOD values ranged between 7.2 - 9.5 mg/l (mean 6.7 mg/l) and pH values varied from 6.0 to 7.0. Water temperature fluctuated between 31.0°C to 34.8°C. Seasonal fluctuations in water temperature distribution play an important role in influencing biological processes. Temperature affects the organisms through direct physiological mechanisms. Temperature related responses vary according to different species and the repercussions for ecosystem dynamics depend upon food web interactions (Kinne, 1963).

Average DO (3.1 mg/l) in the sample of Thane creek was lower than average values reported by Mishra, 2002 (3.9 mg/l) in Ulhas river estuary indicating slight change in water quality due to discharge of small scale industrial effluents.

Lower value of BOD_5 (7.2 mg/l) was observed at sampling station 9/LT with higher value of DO (4.2mg/l) and pH (7.0) at same sampling location, whereas at location number number 14/LT shows higher value of BOD_5 (9.5 mg/l). Higher BOD, low DO and high organic matter in the creek water indicate higher domestic wastewater (sewage) pollution (Somani et al., 2002). This is an indication of the organic pollution (Goldin et al., 2002). These values are within the tolerance limits except DO prescribed under Bureau of Indian Standards for controlling pollution of marine coastal areas (IS: 7967, 1976) and WHO.

Water Quality	WHO Standards	Present Study
Temperature	40°C (5°C)	31.0- 34.8
pH	5.5 - 9.0	6.0 -7.0
Dissolved Oxygen	6 mg/l	2.0 - 4.2
BOD (5 days at 20° C)	30 mg/l	7.2 - 9.5

Zooplankton

The observations on zooplankton density are shown in **Table 2** and presented in **Fig. 2**(a). The zooplankton was represented by 8 Copepoda, 4 Rotifera, 2 each of Echinodermata, Cladocera, Amphipoda, Isopoda and 1 Ostracoda sp. **(Table** 3). The population density of zooplankton ranged from 2,000 to 10,000 No/m^3 in the study area. Generally the low tide samples exhibited

Table 1: Physico-chemical Parameters of the Water of Creek

Sampling Locations	Temperature (°C)	pH	D.O. (mg/l)	BOD_5 (mg/l)
1/LT	33.5	6.8	2.5	8
2/LT	34	6.5	3	8.2
3/LT	31	6.5	2.9	7.6
4/LT	34.2	6	2.8	7.8
5/LT	33.4	6.5	3.2	8
6/LT	32.5	6.7	2.8	8.2
7/LT	33	6.7	3.2	7.8
8/LT	33	6.5	3	8
9/LT	34.8	7	4.2	7.2
10/LT	34	6.8	2.4	8.2
11/LT	33	6.8	2.6	8.2
12/LT	33.5	6.5	3	7.8
13/LT	33.5	6.7	2.8	8.4
14/LT	33	6.5	2	9.5
15/LT	32.5	6.4	2.6	8.4

LT- Low tide, HT-High tide

Table 2: Levels of Zooplanktons Fauna in the water of Thane Creek

Sampling Locations	Total Zooplankton/m^3	% Composition of benthos Group							Shannon Wiener Diversity Index
		Echinodermata	Ostracoda	Cladocera	Rotifera	Copepoda	Isopoda	Amphipoda	
1/LT	8000	-	-	25	-	75	-	-	1.75
2/LT	5000	-	-	-	-	60	40	-	1.52
3/LT	6000	-	33.33	-	-	-	33.34	33.33	1.59
4/LT	5000	40	20	-	-	-	40	-	1.52
5/LT	5000	-	-	40	-	60	-	-	1.52
6/LT	8000	-	-	-	37.5	62.5	-	-	1.56
7/LT	7000	-	-	14.28	-	42.85	42.85	-	1.42
8/LT	8000	-	-	37.5	-	62.5	-	-	1.56
9/LT	4000	60	-	-	-	40	-	-	1.37
9/HT	5000	-	-	-	-	60	20	20	1.5
10/LT	6000	-	-	-	-	83.33	-	16.67	1.45
11/LT	6000	-	50	-	16.66	33.34	-	-	1.46
12/LT	5000	-	-	-	40	60	-	-	2.17
13/LT	6000	33.33	-	-	16.67	50	-	-	1.49
14/LT	10000	-	30	-	-	70	-	-	1.37
14/HT	6000	-	-	16.67	-	83.33	-	-	1.45
15/LT	7000	-	28.56	42.85	-	28.57	-	-	1.56
15/HT	2000	-	-	-	-	71.43	28.52	-	1.54

LT- Low tide, HT-High tide

Ranges of Shannon Wiener Diversity Index (SWDI)

< 1: Indicate maximum impact of pollution or adverse factor

1-2: Indicate medium impact of pollution or adverse factor

>2: Indicate lowest or no impact of pollution or adverse factor

higher density than high tide samples (Kotangale, 1994). Copepoda was dominant group followed by Rotifera, Echinodermata, Cladocera, Amphipoda, Isopoda and Ostracoda. Tiwari and Nair, 2002 reported highest numerical abundance of Copepods contributing to the bulk of biomass in Dharamtar Creek samples with the similarity to the present study. Usually monsoon and postmonsoon months are the most productive period zooplankton from secondary production stand point (Menon et al., 1977) and often exhibit higher diversity index value (Srinivasan and Santhanam, 1991). However, higher species diversity value was recorded in summer as compared to premonsoon and monsoon seasons at Coleroon estuary (Jagadeesan and Ayakkannu 1992). Tolerance of Rotifers to the higher salinity was observed by (Somani, 2002). The values of diversity index in the Thane creek ranged between 1.37 - 2.17 (average 1.77). Thus the index value of zooplankton shows moderately polluted nature of water of the creek in the study area.

Meiobenthos

The observations on meiobenthic fauna are shown in **Table 4** and presented in **(Fig.2(b)**. Meiobenthic fauna was represented by 15 Foraminifera, 10 each of Ostracoda, Polychaeta and 2 Nematode **(Table 5)**. It was dominated by Foraminifera. However, at sewage and industrial effluent discharge point, the Foraminifera and Ostracoda were observed higher number of meiobenthos. The meiobenthic faunal counts ranged between 21,600 - 1,34,400 No/m². This count was observed minimum at locations of 9/LT, 10/LT and 12/LT showing the impact of effluent discharge from small industrial activities. Higher value of benthos count (134400/m²) was observed at a location of 14/LT. These values of benthos count may be due to the nutrient enrichment due to

Fig.2: Faunal dominance in the Thane Creek, Mumbai

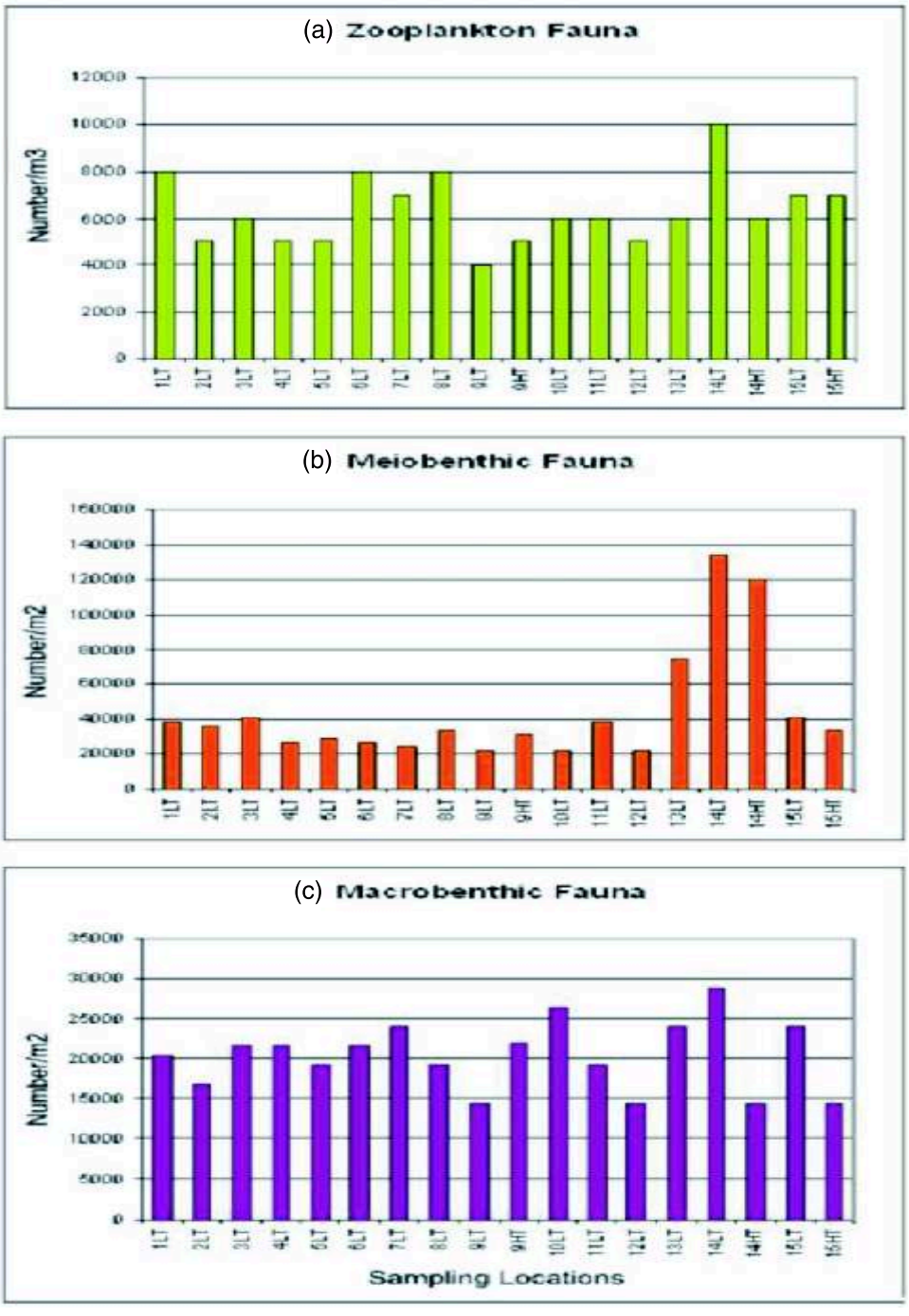

Table 3: Zooplankton Faunal species observed during study period

Sr. No.	Family	Meiobenthos Species
1	**Copepoda**	*Eucalanus nauplius.*
		Oithana nana
		Horpacticoid sp.
		Nauplius sp.
		Labidocera laevidentata
		Acardia spinata
		Miracia efferata
		Ectinosoma dentatum
2	**Rotifera**	*Encentrum sp.*
		Brachionus sp.
		Asplanchana sp.
		Filinia sp.
3	**Echinodermata**	*Bipinaria larva*
		Auricularia sp.
4	**Amphipoda**	*Primno macropus*
		Elasmopus rapax
5	**Isopoda**	*Eurydice pulchra*
		Stenetrium stebbingi
6	**Cladocera**	*Penilia avirostris*
		Evadne tergestina
7	**Ostracoda**	*Ostracod sp.*

organic contents in the water. The maximum impact of benthos was observed at locations (9/LT) at a wastewater discharge point. Values of diversity index ranged from 1.789 to 2.529 (Avg. 2.159) indicates medium impact of pollution with a medium adverse factor. Meiobenthos were dominated more in the sediment depth of 4-6 mm and decreased progressively in the deeper layers showed patchy dispersion, related to the availability of food and predation (Ansari and Ingole, 1983). Setty, 1976 observed abundance of Foraminifera population in polluted water of Cola Bay, Goa.

Macrobenthos

The observations on macrobenthic fauna are shown in **Table 6** and presented in **(Fig. 2(c)** The count of macrobenthos ranged from 14,400 to 28,800 No/m². Macrobenthic fauna was represented by 15 Gastropoda, 3each of Bivalves, Polychaeta and Podocopida **(Table 7)**. The dominated species was observed as Gastropoda. The benthos count was reduced at the locations of locations (9/LT, 12/LT, 14/HT and 15/HT showing the impact of effluent discharge in the area. Higher value of benthos count (28800/m²) was observed at station 14/ LT. Higher value of benthos count may be due to the nutrient enrichment of organic contents (Mishra, 2002). Bivalves was dominant group at Dahanu, Danda and Savta Creek in west coast of India (Kotangale, 1994), it was replaced by Gastropoda. The values of diversity index ranged from 0.919 to 2.168 (mean 1.543). It indicates medium impact of pollution showing the medium adverse impacts.

Enrichment of coastal waters due to riverine flow and land runoff also seems to be one of the factors contributing to richness of fauna in nearshore regions

Table 4: Levels of Meiobenthic Fauna in the Water of the Creek

Sampling Locations	Total Benthos/m²	% Composition of benthos Group				Shannon's Weinner Diversity Index
	(Meiobenthos)	Foraminifera	Ostracoda	Polychaeta	Nematoda	
1/LT	38,400	56.25	31.25	12.5	-	2.529
2/LT	36,000	86.66	-	13.34	-	2.019
3/LT	40,800	70.58	29.42	-	-	2.254
4/LT	26,400	63.64	27.27	0.09	-	2.114
5/LT	28,800	83.34	8.33	8.33	-	2.082
6/LT	26,400	72.72	18.18	0.09	-	2.227
7/LT	24,000	80	-	10	10	2.168
8/LT	33,600	78.58	-	21.42	-	2.071
9/LT	21,600	77.77	22.23	-	-	1.974
9/HT	31,200	61.55	23.07	15.38	-	2.614
10/LT	21,600	66.66	11.12	11.11	11.11	2.112
11/LT	38,400	68.75	-	31.25	-	2.054
12/LT	21,600	66.66	22.23	11.11	-	2.085
13/LT	74,400	80.64	16.12	3.24	-	2.138
14/LT	134400	91.07	5.35	3.58	-	2.106
14/HT	120000	86	14	-	-	1.789
15/LT	40,800	70.58	29.42	-	-	2.254
15/HT	33,600	78.58	-	21.42	-	2.071

Table 5: Levels of Meiobenthic Fauna in the Water of the Creek

Sr. No.	Family	Meiobenthos Species
1	**Foraminifera**	*Ammonia sp.*
		Spirolina orietina
		Triloculina sp.
		Elphidium sp.
		Amphistegina sp.
		Textularia sp.
		Bolivina sp.
		Rosalina sp.
		Globiogerina sp.
		Rotalia sp.
		Quinqueloculina sp.
		Clavulina sp.
		Cyclogyra sp.
		Cibicides sp.
		Nonion sp.
		Macrocyprina sp.
		Calcarina sp.
		Thalossocypria sp.
2	**Ostracoda**	*Cytherelliodea sp.*
		Paranesidea sp.
		Cyprideis sp.
		Triangulocypris sp.
		Propontocypris sp.
		Halocypris sp.
		Polychaete larva
3	**Polychaeta**	*Perinereis sp.*
		Scolelepis squamata
		Nectochaeta sp.
		Halosydna sp.
		Halosydna sp.
		Arabella mutans
		Polyophithalmus sp.
		Aktedrilus sp.
		Marlonina
		Spiopeltiboneae sp.
4	**Nemotoda**	*Tricoma hopperi*
		Paramonohystera sp.

Table 6: Levels of Macrobenthic Fauna at Thane Creek, Mumbai, MS

Sampling Locations	Total Benthos/m² (Mcrobenthos)	% Composition of benthos Group				Shannon's Weinner Diversity Index
		Gastropoda	Bivalves	Polychaeta	Podocopida	
1/LT	20,400	25	75	-	-	2.114
2/LT	16,800	71.42	28.58	-	-	1.556
3/LT	21,600	-	77.77	22.23	-	1.974
4/LT	21,600	33.33	66.67	-	-	1.225
5/LT	19,200	25	50	-	25	1.75
6/LT	21,600	22.23	77.77	-	-	1.974
7/LT	24,000	50	50	-	-	1.76
8/LT	19,200	-	62.5	37.5	-	1.299
9/LT	14,400	11.11	66.66	22.23	-	0.919
9/HT	22,000	20	60	20	-	2.168
10/LT	26,400	18.19	81.81	-	-	1.494
11/LT	19,200	-	62.5	37.5	-	1.299
12/LT	14,400	16.66	50	33.33	-	1.458
13/LT	24,000	-	50	25	25	1.522
14/LT	28,800	41.66	58.33	-	-	1.958
14/HT	14,400	11.11	66.66	22.23	-	0.919
15/LT	24,000	50	50	-	-	1.76
15/HT	14,400	16.66	50	33.34	-	1.458

Table 7: Macrobenthic Faunal species observed during study period

Sr. No.	Family	Macro-benthos Species
1	**Gastropoda**	*Planaxis lineatus*
		Melanella intermedia
		Bermudoclis bermudensis
		Modulus modulus
		Nassarius nevilliana
		Eunaticina pomotiella
		Rhodope sp.
		Natica tigrina
		Janthina pollida
		Litlorina sp.
		Hlydatina velum
		Batiilaria sp.
		Litiopa sp.
		Oliva sp.
		Morula sp.
2	**Bivalves**	*Perna sp.*
		Meretrix sp.
		Tellina sp.
3	**Polychaeta**	*Dontosyllis enopia*
		Pontodrillus bermudensis
		Arenicola cristata
4	**Podocopida**	*Paronesidea sp.*
		Cyprideis sp.
		Tholossocypria sp.

According to Patil et al., (1975 and 2002) the availability of food and the associated chemical changes influence the population dynamics. Thus the diversity index value for zooplanktons and zoobenthos shows moderately polluted nature of water of the Thane Creek.

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Studies on Spermatogenic Damages in Squirrel Testis Induced by Anti-cancer Agent Cyclophosphamide

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Abstract : A commonly used anticancer drug, Cyclophosphamide (CPA) is a bifunctional alkalyting agent, a well known male-mediated developmental toxicant with clear stage-specific effects on male germ cells. Present experiments were conducted to observe cellular toxicity on squirrel testis after 15 and 30 days Cyclophosphamide administration intraperitoneally (ip). Total 12 adult male Indian palm squirrels, *Funambulus pennanti* (Wroughton) were divided into one control and two experimental groups containing 3 squirrels in each group. Experimental groups received CPA in two different doses i.e. 6 and 12mg, whereas control group received same amount of normal saline, intraperitoneally. The 6mg dose treatment resulted into an insignificant and 12mg dose treatment into significant changes in the body weight ($P<0.05$) and testicular weight ($P<0.05$) and many untoward behaviour. Similarly decreased diameter of seminiferous tubules ($P<0.05$) followed by an increase in interstitial spaces. The affected seminiferous tubules also exhibited atrophy, exfoliation and hence depletion of germinal elements. The other toxicological effects on histoarchitecture of testis were an increase in the thickness of tunica albuginea and disruption of lamina propria, loss of circular contour of seminiferous tubules. Of interest was hydropocity of seminiferous tubules due to accumulation of fluids resulting from cytolysis of various germinal elements. The various stages of primary, secondary spermatocytes, spermatids, the Sertoli and Leydig cells were altered significantly compared to controls, spermatids exhibited abnormality in shape. All above mentioned histopathological changes were in dose dependent manner. From the foregoing finding it can be concluded that these qualitative and quantitative changes in male gonads may alter the reproductive performance of animals.

Key words : Spermatogenesis, Sertoli cell, Leydig cell.

Introduction

Gonad is an important component of reproductive system, associated with series of cellular interactions, differentiation to form mature germ cells through process of spermatogenesis and any insult at this stage on gonads may impair fertility. Treatment with cytotoxic chemotherapy is associated with significant gonadal damage and alkylating agents are the most common agent implicated in the development of infertility (Schilsky, 1980; Pont and Albrecht, 1997; Shetty and Meistrich, 2005; Vaisheva et al., 2007). The chemotherapeutic drug Cyclophosphamide (N, N-bis (2-choroethyl)-2-oxo-1-oxa-3-aza-2u {5}-phosphacyclohexan-2-amine) is one of the cytotoxic alkylating agent. Its cytotoxic effects are the result of chemically reactive metabolite that creates DNA adducts, DNA-DNA and DNA-protein cross links, sister chromatid exchanges, chromosomal aberration and DNA strand breaks in many cell types, including germ cells (Sotomayor and Cumming, 1975; Bishop et al.,1997; Condrington et al., 2004). The goal of present study is to elucidate impact of Cyclophosphamide treatment on male germ cells which directly play an important role in male reproductive performance of animal.

Materials and Methods

Animals and treatments

Adult male Indian palm squirrels weighing between 100 to 150g were trapped alive in and around Nagpur City during the breeding period from January to July 2007 (Reddi and Prasad, 1968). After a week of acclimatization to laboratory condition, all squirrels were administered intraperitoneally Cyclophosphamide using one of the two schedules i.e. low chronic dose of 6mg/ KgBW for 30 days and high chronic dose of 12mg/KgBW for 15 days dissolved in saline. The controls also received the same amount of saline (Tables-1 and 2).

The animals were sacrificed using chloroform 24 hours after the last day of each experiment. For light microscopic study the testis was excised immediately

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Table 1 : Experimental Design for Low chronic dose Cyclophosphamide treatment

Number of animals and sex	Treatment	Dose (mg/KgBW/day)	Route	Duration
3 males (Experimental)	Cyclophosphamide	6 mg chronic	I.P.	30 days
3 males (controls)	Saline	Equal volume	I.P.	30 days

Table 2 : Experimental Design for High chronic dose Cyclophosphamide treatment

Number of animals and sex	Treatment	Dose (mg/KgBW/day)	Route	Duration
3 males (Experimental)	Cyclophosphamide	12 mg chronic	I.P.	15 days
3 males (controls)	Saline	Equal volume	I.P.	15 days

I.P. = Intraperitoneal, BW = Body Weight

and fixed in Bouin's fixative, dehydrated in ethanol and embedded in paraffin wax. The sections cut at 5µm were stained with haematoxylin and eosin. Measurements were taken with an occular micrometer wherever essential.

Statistical Analysis

To indicate individual variations in each corresponding region, the mean values and standard deviation (mean ± SD) for measurements from three animals were calculated. The statistical significance of differences for these values in different regions was assessed using 't-test' (Delgaard, 2008). A significant level of $P<0.05$ was accepted.

Results

Both the treatment resulted in the suppression of body weight (fig.1) and testicular weight (fig.2) being

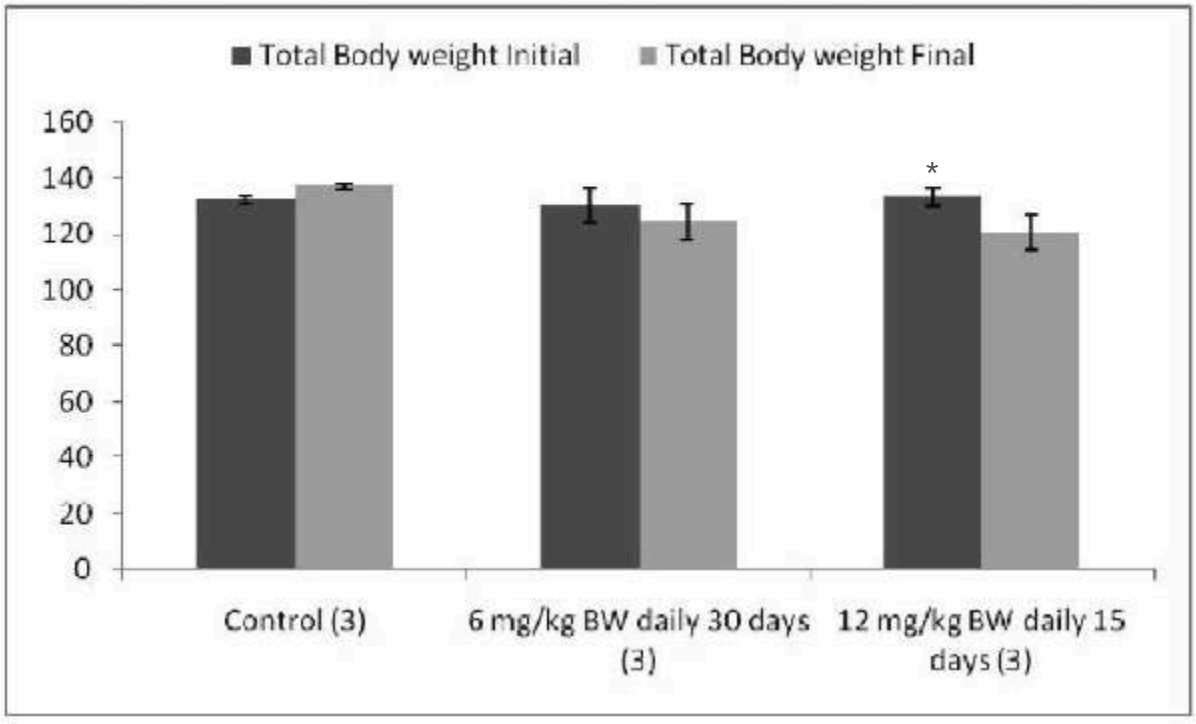

Fig. 1 : Body weight changes over the course of 30 and 15 days treatment with Cyclophosphamide, expressed as weight on the last day of treatment divided by weight on the first day of treatment (Control n=3, Cyclophosphamide n=3). Treated squirrels did not gain as much weight as the control squirrels. *P<0.05.

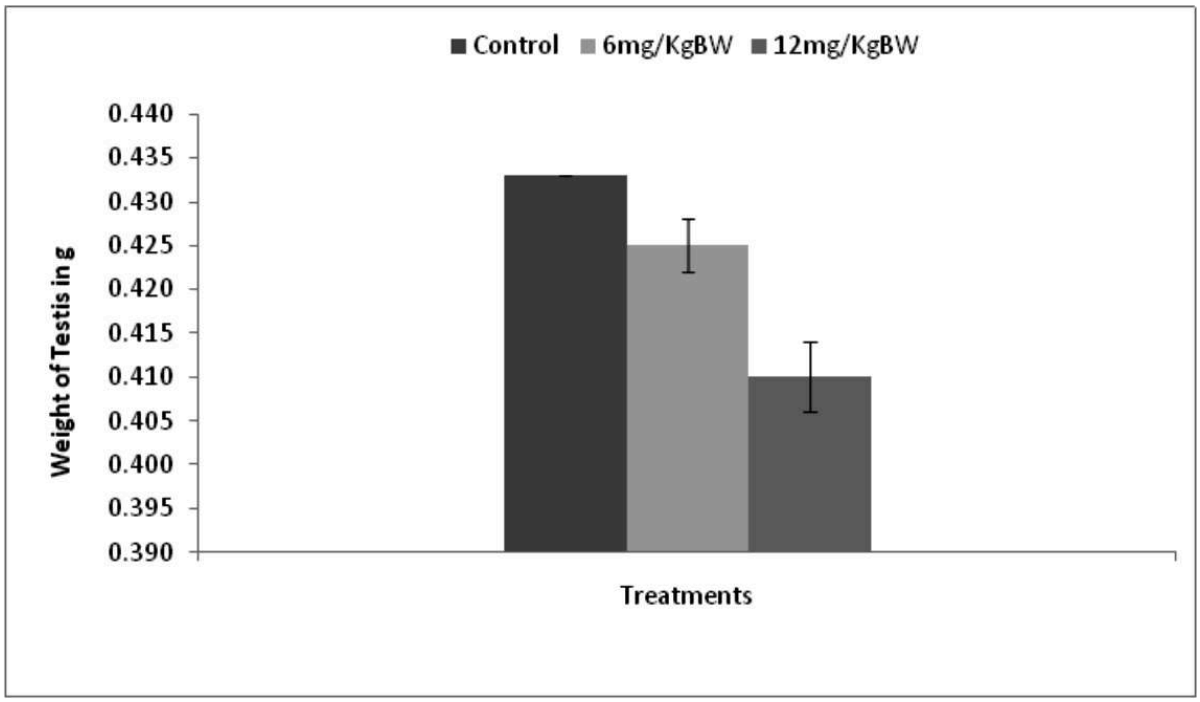

Fig. 2 : Weight of testis after 30 and 15 days treatment with vehicle or Cyclophosphamide (Control n=3, Cyclophosphamide n=3). There was a significant decrease in the weight of the testis of the Cyclophosphamide treated animals. *P<0.05 and **P<0.01.

significant for higher dose (P<0.05). The other behavioral changes were sluggishness, loss of appetite, withdrawn mood, however, mortality rate was zero percent. Similarly testis revealed smallness in size, irregularity in general contour, bizzare appearance of spermatic arteries supplying blood to the testis.

Vehicle- treated controls

The histological sections of testis showed elongated to circular seminiferous tubules covered with moderately thick lamina propria. The germinal epithelium showed two types of cells (1) the Sertoli and (2) germinal. Sertoli cells formed a frame around the developing germ cells. The germ cells were stacked in 4-8 layers. All stages of spermatogenesis were observed. Leydig cells were round or polygonal with central nuclei (fig.3).

Chronic low dose treatment (6mg/KgBW/day)

Evaluation of testicular histopathology revealed that the drug has implicated different degrees of damages mostly in the peripheral than the central seminiferous tubules manifesting an increase in thickness of tunica albuginea. Seminiferous tubule diameters were decreased by 20% (fig.11), resulting into an increase in the intertubular spaces of various sizes prevailing in between almost 90% tubules, simultaneous decrease in triradius mesenchymal area, displaced lamina propria, partial alteration in morphology of Leydig cell with reduction in diameter, appearing less in frequency, granulation of cytoplasm and condensation of central nuclei, blood capillaries supplying to triradius mesenchyme appeared totally normal (figs.4 and 5). The cellular proliferation of gonads were also affected, thus a decrease in cellularity due to apoptosis of germ cells coupled with a reduction in the height of seminiferous epithelium and partial inhibition of spermatogenesis probably at round spermatid or pachytene stage of spermatocyte leading either to oligospermia or normospermia, disorganization of Sertoli cells, accommodating few long spermatids either their breakdown or extreme regression, detachment from the basement membrane, bilateral compression, pyknosis and vacuolation of nuclei, ballooned or highly vacuolated cytoplasm, residual cytoplasm appeared reticulated. Similarly pachytene spermatocytes, spermatogonia also appeared either condensed, pyknotic or vacuolated (fig.5).

Chronic high dose treatment (12mg/KgBW/day)

Treatment induced disturbances in the histology of the seminiferous tubules varying from small vacuoles in the epithelium to total loss of germ cells. Similarly

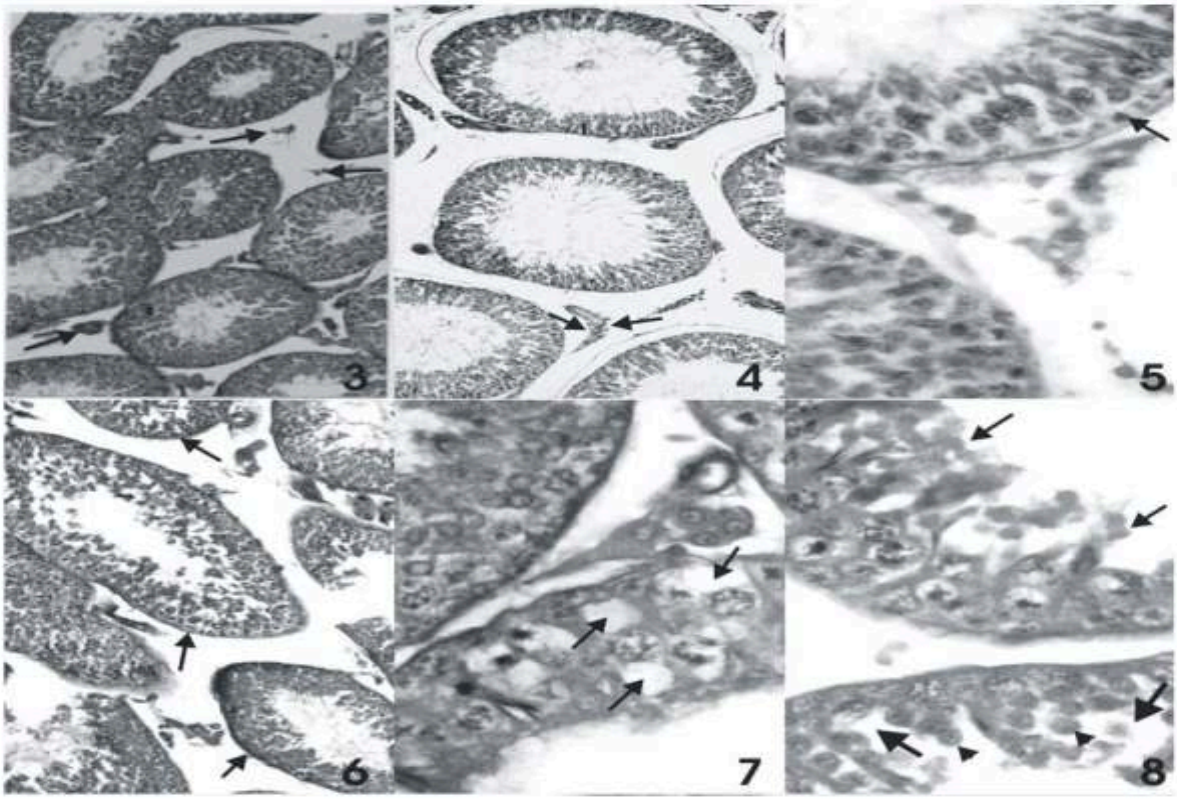

Fig. 3: Seminiferous tubules from vehicle-treated control squirrel testis. Note different stages of spermatogenesis, well developed Sertoli cells and Leydig cells (arrow) X100.

Fig. 4: Few seminiferous tubules from 6mg/KgBW chronic low dose. Note partial shrinkage,displaced lamina propria, an increase in the intertubular spaces, decrease in triradius mesenchymal area (arrow). Different degrees of damage implicated all over the cross-section, exhibiting hypotropic epithelium and intra-epithelial vacuolation X 100.

Fig. 5: Part of seminiferous tubules from 6mg/KgBW further magnified. Note detachment of lamina propia, germ cells remarkably depleted and sperms are few, spermatogonia displaying pyknosis (arrow), regressed Sertoli cells, compressed or broken into pieces, loss of cytoplasm, with vacuolated large-sized pyknotic nuclei, ballooned or highly vacuolated cytoplasm. Residual cytoplasm appears reticulated, highly reduced mesenchye with vacuolated Leydig cells appearing less in frequency and partially atrophied as their nuclei show condensation X400.

Fig. 6: All the tubules (12mg/KgBW) display disorganized cellular associations corresponding to different stages of the seminiferous epithelial cycle (arrow). Note dimunation in size of seminiferous tubules and hence a remarkable increase of interstitial spaces. Note wavy, displaced and thickened peritubular membrane and low cellular population. The degree of vocuolation and loss of germinal epithelium is variable in all tubules shown. Intraepithelial large vacuolation with cellular debris suggests apoptosis X 100.

Fig. 7: Two seminiferous tubules enlarged to illustrate atrophy of testicular germ cells and hence formation of large vacuoles (arrow). Leydig cells reveal partial alteration. Detachment and killing of many stem cells, spermatogonia show pyknosis. Basement membrane show wavy lamellae and infoldings enveloping the tubules, sometimes hanging into loops X 400.

Fig. 8: Two seminiferous tubules demonstrating extreme histopathological changes with 12mg/KgBW. Note denudation with reduction in the number of myoid cells as well as low cellular population density of spermatogenic elements, extreme shrinkage of spermatogonia. Disorganization of Sertoli cells with sloughing off of apical tip cytoplasm in the lumen (arrow), pyknosis and vacuolation of the nucleus, remarkable reduction in the number of long spermatids, however, sperms are not discernible. Extreme condensation, fragmentation orgranulation of chromatin material as well as vacuolation in the pachytene spermatocyte. Round spermatids exfoliated into adluminal compartment of the tubules (arrow head). Extreme Prevalence of large spaces (thick arrow) in between the extremely regressed Sertoli cells suggesting high frequency of apoptosis of germinal epithelium X 400.

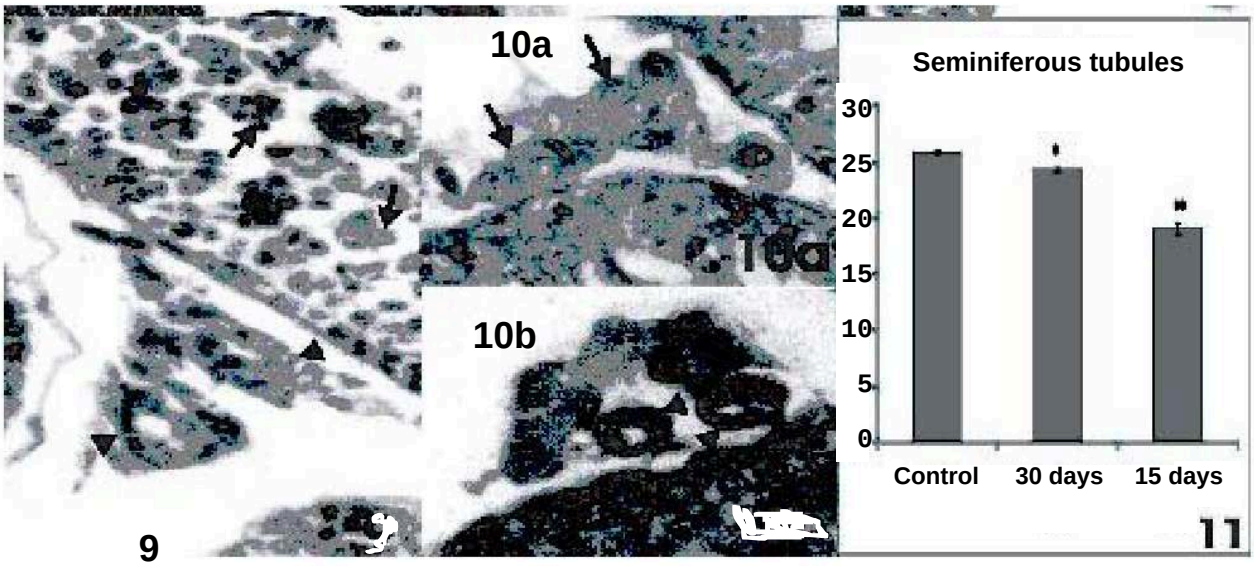

Fig. 9: Part of the seminiferous epithelium photographed, staging of spermatogenesis could not be evaluated due to the dramatic damage to tubules (arrow), however, stem cells are discernible (arrow head). Note congestion of blood capillaries (arrow head) with atrophied Leydig cells X 400.

Fig.10 : a, 10 b: Leydig cells from 12mg/KgBW/day treatment. Demonstrating atrophy of some cells (arrow), similarly remarkable thickening of perivascular tissue (arrow head) of the blood capillary X 1000.

Fig. 11: Diameter of seminiferous tubules of squirrel testis after 30 and 15 days treatment of Cyclophosphamide (Control n=3, Cyclophosphamide n=3). There was a significant decrease in the diameter of seminiferous tubules of the Cyclophosphamide treated animals. *P<0.05 and **P<0.01.

lamina propria appeared wavy, displaced and remarkably thickened, sometimes complete denudation of the tubule with reduction in the number of myoid cells. Although the extent of damage was variable, a mean of 50% of seminiferous tubules were heavily damaged (fig.6). Damage ranged from the presence of small epithelium to tubules deprived of spermatocyte and spermatids and was accompanied by an increased incidence of germ cell apoptosis (figs. 8 and 9). In some tubules staging spermatogenesis could not be evaluated due to the dramatic damage (fig.9). Similarly some tubules contained round spermatids with the acrosomic head caps of spermatogenesis stage VI, some did not have the second generation of round or elongated spermatids (fig.8). Bilateral compression of Sertoli cells with apical sloughing and shedding of cellular material and therefore accumulation of remnants of residual cytoplasm (fig.8). Thus the high dose treatment revealed the similar but enhanced changes implicating low frequency of germinal lineage cells in some tubules due to frequent apoptosis, however, some tubules survived damage. The extensive widening of the intercellular spaces around the Sertoli cells due to loss of spermatogenic elements exhibited "partial Sertolization" of the tubules (figs. 7 and 8). Similarly the Sertoli cells exhibited severe structural alteration due to disruption of tight contacts with neighbouring cells or by severe disintegration of their apical portion tipped off. Of interest was hydropocity of seminiferous tubules due to accumulation of fluids resulting from cytolysis of various germinal elements. The surviving stages in some of the tubules were

oligospermic or oligozoospermic condition, occurrences of numerous necroses, abnormal mitotic figures of spermatogenic elements, malformed spermatids and spermatozoa with various degrees of "coiling of tails". of Leydig cells displayed moderate alteration and remarkable thickening of perivascular tissue of the blood capillary (figs. 7, 8, 10a and 10b).

Discussion

Animals in reproductive age can be exposed to several side effects when chemotherapeutic agents are administered for treatment. Reduction in the body weight from moderate to severe has been recorded (Velez *et al.*, 1989; Elangovan *et al.*, 2006), on the contrary Takeda *et al*., 1985 recorded no significant reduction. Correlative to body weight a decline in the circulating blood serum androgen have been noticed

since androgen are a potent stimulant of nitrogen retention (Bhasin *et al.,* 1997) or an increase in potassium concentration (Turner and Bagnara, 1976) which maintains the body weight. Our results are also in accordance with Trasler *et al.,* 1986; Watanabe *et al.,* 2000; Kenney *et al*., 2001 who described Leydig cell atrophy as well as reduction in LH levels needed for the synthesis of testosterone. Similarly a reduction in the weight of testis, lower testicular volume points to reduced androgen levels (Kresurer *et al.,* 1987) and hence an increase in androgen binding protein (Buchanan and Riches,1986), however, in the present study testosterone levels were not measured. The side effects were further manifested by histopathological changes in the testis such as extreme reduction in germinal epithelium due to apoptosis, reduction in triradius mesenchyme, oligospermia or oligozoospermia. Decrease in sperm production thus correlate well with decrease in testicular weight (Robaire *et al.,* 1979), flaccid appearance and gross regression of scrotum, reduced blood supply in the high dose treatment also support the above statement since any reduction in the testicular blood flow due to toxic effect of CPA causes reduction in the circulating testosterone and on the spermatogenesis (Papadakis *et al*., 1999; Kenney *et al*., 2001). At the same time CPA being a potent inhibitor of testicular 3á-hydroxysteroid oxidoreductase activity, itself binds to the catalytic binding site of the substrate like DHT (5á-dihydroxytestosterone) thus reducing the ABP production which would have helped in the maintenance of the testis weight as well as histological architecture. Our result are support by Hales *et al.,* 2005; Vaisheva *et al.,* 2007.

From the forgoing it is concluded that the histopathological changes in the testis reflect the toxic status of Cyclophosphamide due to its antimitotic, antiandrogenic, antigonadotropic and antispermatogenic properties which are dose and duration dependent beside being toxic.

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Overview on Social Impacts of Special Economic Zone (SEZ) in India

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Abstract : The present study aims at examining the social impacts of Special Economic Zone (SEZ). It identifies three channels through which SEZ addresses issues such as employment generation, skill formation (human capital development), technology and knowledge up- gradations etc. Findings reported in the study are based on the data collected from both secondary sources and primary surveys. The primary data was generated through extensive interviews. It assesses the economic benefits to community and social impacts due to SEZ activities. The analysis reveals that employment generation has been the most important channel through which SEZ lend themselves to human development concerns. Besides, working conditions, non-monetary benefits such as transport, health and social security are better than rather limited.

Key words : Technology transfer, Employment generation, Social security benefits, Poverty reduction

Introduction

India, the largest democracy, with population exceed of 1 billion, presents the worlds biggest middle class consumer market of 300 million people. It has a vibrant manufacturing and service sector. The country, with ready access to South-East Asia, East Asia, the Middle East and Africa provides good opportunities in infrastructure development. The Special Economic Zones (SEZ) has been conceived with a view to provide an internationally competitive and hassle free environment to develop world-class infrastructure for production and exports.

SEZ is a geographical region that has economic laws that are more liberal than a country's typical economic laws. The category 'SEZ' covers a broad range of more specific zone types, including Free Trade Zones (FTZ), Export Processing Zones (EPZ), Free Zones (FZ), Industrial Estates (IE), Free Ports (FP), Urban Enterprise Zones (UEZ) and others (ILO, 1998); ICFTU, 2004; PRIA, 2000; Hossain, 2001; Mazumdar, 2001 and Kemal, 2001).

Usually the goal of an SEZ structure is to increase foreign investment. One of the earliest and the most famous SEZ were founded by the government of the People's Republic of China under Deng Xiaoping in the early 1980s. The most successful SEZ in China has developed from a small village into a city with a population over 10 million within 20 years. (Ali, 2000). An SEZ is a trade capacity development tool, with the goal to promote rapid economic growth by using tax and business incentives to attract foreign investment and technology. Today, there are approximately 3,000 SEZs operating in 120 countries, which account for over US $ 600 billion in exports and about 50 million jobs. By offering privileged terms, SEZs attract investment and foreign exchange, spur employment and boost the development of improved technologies and infrastructure.

The Special Economic Zone Act, 2005 came into force with effect from February 2006, with SEZ rules legally vetted and approved for notification. The SEZ rule provides the simplification of procedures for development, operation, and maintenance of the SEZs and for setting up and conducting business in SEZs. This includes simplified compliance procedures and documentation with an emphasis on self-certification; single window clearance for setting up of an SEZ, setting up a unit in SEZ and clearance on matters relating to Central as well as State Governments (Agrawal, 2007). The Government of India in April, 2000 announced the introduction of Special Economic Zone policy in the country, deemed to be foreign territory for the purpose of trade operations, duties and tariffs. As of 2007, more than 500 SEZs have been proposed, 220 of which have been created. This has raised the concern of the World Bank, which questions the sustainability of such a large number of SEZs.

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Today's Special Economic Zone is growing on such an alarming rate that the view of tomorrow will be very different as compared to today because their will not be any employment problem or any such problem which will stop the person to go ahead in his life with all the modem techniques etc.

In India Andhra Pradesh and Maharashtra are on the top of the List of number of SEZs in the country. The number of SEZ approved for Maharashtra are 39, Andhra Pradesh 29, whereas for Tamil Nadu 20, Karnataka 19, Gujarat 13 and Haryana 11.

States with most 'in principle' approvals: Maharashtra: 24, Haryana: 23, Karnataka: 15, Uttar Pradesh: 9, Gujarat, Punjab and Tamil Nadu 7each More than 25000 people have been displaced due to Special Economic Zone

26, 800 hectares lands are acquired for approved Special Economic Zones 75,000 hectares may be acquired for 'in principle' and 'under consideration' Special Economic Zone. (The Rally, 2007)

Materials and Methods

List of SEZs in India

It was also envisaged that some of the existing Export Processing Zones would be converted into Special Economic Zones. Accordingly, the Government has converted following Export Processing Zones located at

Kandla and Surat (Gujarat)

Cochin (Kerala)

Santa Cruz (Muinbai-Maharashtra)

Falta (West Bengal)

Chennai (Tamil Nadu)

Visakhapatnam (Andhra Pradesh)

Noida (Uttar Pradesh)

Nanguneri and Tirunelveli (Tamil Nadu)

SEZ Policies

The SEZ Policy was first introduced in India in April, 2000 as a part of the Export Import (EXIM) policy in India. The policies are provides for setting up of SEZ' in the public, private, joint sector or State Governments. *(Special Economic Zones "An Indian Perspective, 2007)*

SEZ policy and some important part of it are given below:

a) The SEZs in India are of three types:

   1. Multi-product SEZs occupying minimum 1000 hectares of land (on 5th April, 2000 the maximum limit of these zones is earmarked as 5000 hectares); may produce garments and automobiles.
   2. Sector-specific SEZs occupying minimum 100 hectares of land, e.g., garment, leather, electronics-SEZs etc.
   3. Gems and Jewellery, IT-ITeS-BPO and Biotech-SEZs occupying minimum 10 hectares of land (may be reduced to 4 hectares in special cases). Backward states have options of relaxation of minimum criteria of land. (Taking 1 hectare -nearly 2.5 acre, one can comprehend the real size of the SEZs.

b) The country is divided into two territories; one of which is SEZ and another is 'Domestic Tariff Areas (DTAs). The area outside of the SEZs is DTAs where the laws of the country will be applicable. On the other hand, in the SEZs the laws and courts of the country may be applicable only partially. In fact, the SEZs will enjoy special laws. The Act on SEZs clearly states: "An SEZ... is like a foreign territory within a country"

c) Private developers can build the SEZs and fabulous incentives will be provided for them. Even the local contractors / promoters can also enjoy the same benefits. 'Processing units' set up in the SEZs will also enjoy several incentives / concessions. The state governments can build SEZs themselves according to their wish

d) Minimum 35% of SEZs must be 'processing area' (i.e., industry / factory / projects including infrastructures). Rest of the 65% area will be provided for developing housing complexes, hotels, restaurants, hospitals, shopping malls, entertainment centers, multiplexes, playgrounds, and even golf courses, etc

e) It was decided earlier that the developers / promoters would be provided land of any sizes by the state governments. Land will be acquired by Land Acquisition Act (1894) of British colonial era. Under the protests developing all over the country, it is declared that the developers will have to buy land their own.

f) The (fiscal) incentives given to the developers of the SEZs are as follows: The developers will get income-tax exemption for a block of 10 years in 15 years at the option of the developers; Imports / domestic procurement of goods for development, operation and maintenance will be duty-free; Exemption from service tax. The (fiscal) incentives given to the industries / enterprises operating within SEZs: 100% income tax exemption for a block of five years, 50% tax exemptions for two years; exemption from excise duty on procurement of capital goods, raw materials, consumable spares, etc. from the domestic market; exemption of import duties on the same if exported from abroad without any license or specific approval; exemption from service tax, state sales tax, octroi, mandi tax, turnover tax, and other duties / cess or levies on the supply of goods from DTAs.

g) Other incentives: 100% FDI in manufacturing sector through automatic route; exemptions from industrial licensing for manufacture of items reserved for small sector industries; full freedom for subcontracting, including subcontracting from abroad and DTAs. The area incorporated in the proposed SEZs is free from environmental restrictions profits allowed to be repatriated freely without any dividend balancing requirement water, electricity and other services would be provided as required private generation, transmission and distribution of power in SEZs allowed; Developers are even permitted to build ports, airports, roads etc. at their requirement.

h) The goods sold by SEZs in DTAs will be regarded as imports in the DTAs. An example: Reliance Industries set up a new refinery in Jamnagar SEZ (Gujarat) and that could end up 'exporting' bulk of its output in DTAs (or India). Hence, the people of India (DTAs) have to cough up import duties to buy the Reliance petrochemical products

i) In these SEZs' all the units will be declared as 'Public Utilities' where existing labour laws will not act. Besides this, the state governments can act new labour laws for the respective state-SEZs which were already done in Maharashtra, Gujarat, Tamil Nadu and UP. Hire-and-fire, employing casual and/ or contract labour under any conditions will be allowed. The workers/employees are stripped off the rights of strike.

j) A 'Development Officer' will govern the SEZs. This non-elected officer will govern each inside-affair of the SEZs including the municipal, labour etc. According to a bourgeois analyst, "democracy" or "bureaucracy" nothing will be applicable here. The law of the 'mainland' India may be applicable partially. In fact, there are no words about any criminal courts in the SEZ Acts though provisions of civil courts arc present. A special security force will look into the internal security of the SEZs. It is not clarified whether the police forces of 'mainland' India can interfere here.

Results and Discussion

Man is the vital element in any geographical area of a region. Population makes the resources of a region meaningful as it determines and fulfils all human needs. Include all the social aspects in the region such as population, infrastructure resources, health, education, employment, economy of the region and cultural and heritage etc. Social environment includes the life style of the people, their culture and their community. SEZ play a central role of employment in poverty reduction. It identified three channels through which SEZ may affect social capabilities.

Social Impact

I) Employment Impact

The employment impact of SEZs operates

through three channels: (1) Direct employment for skilled and unskilled labours (2) Indirect employment and (3) Employment for women workers

Direct Employments

Due to the availability of labour at low wages, developing countries generally attract investment into simple processing labour intensive industries. Increased demand of unskilled labour with SEZ. Higher value added activities as SEZs grow, increase in demand for skilled labour also. SEZs also generate employment for unskilled labour by creating physical infrastructure within the zone. This stimulates the local construction industry giving employment for unskilled labour (Sivalingctm, 1994). This is a substantial impact on employment generation

Indirect Employments

The indirect impact is ancillary employment opportunities generated in sector of the economy affected by the operations of the SEZ. These include: transport, communication, tourism, hospitality, banking and insurance. Employment opportunities generate for both skilled and unskilled labour.

There are other three channels through which SEZ generate impact on employment generation, (1) SEZ generates developmental funds, which facilitate generation of economic activities and employment, (2) SEZ generates economic activity outside the zone due to the transformation of investment fund and purchase of inputs and services from the rest of economy and (3) there is an increase in demand for various goods and services such as housing, education, health, and transport. This in turn has multiplier impact on human development.

Employment for Women

Evidence suggests that women's share in total employment in SEZs is substantially higher than both the economy as a whole as well as the manufacturing sector outside the SEZs. (Kusago and Tzannatos, 1998), SEZ arc. thus expected to contribute substantially to the improvement of women. SEZ contributed to human development by increasing women employment opportunities. (Madani, l999). The implicit assumption is that job creation alleviates. Unemployment, generates income, improves standard of living and results in social development and poverty reduction.

II) Skill Formation Impacts

There are various modes, SEZ contribute to skill formation. One is the firm level activity whereby the host country labour force acquires skills from within the firm through training and learning by doing on the job (Kusago and Tzannatos, 1998), SEZ can directly impact the skill formation. UK workers are provided additional training on and off the job. Second method involves up jading of the education system to cater to the need of the zone units. In the Shenzhen SEZ (China), Shrilankan SEZs and Mexican maquiladoras institutes are established to improve technical and vocational skill of worker in the Zone. These programmers aim at providing technical education in the zones. SEZ units may also be setting up training institutes to impart training to the labour to create the relevant pool of skilled labour.

Skill formation for the poor unskilled women's also occurs, through assimilation of industrial discipline. They increase the welfare of pool unskilled worker by increasing the range of job opportunities available of them. Improve skilled and productivity increase workers, income earning capacity. In the long term, the creation of a macro environment in which return to education and skill development arc high is an important component of the skill formation impact of SEZ. That can play a crucial role in upgrading domestic entrepreneurial skills.

III) Technology Upgrading Impact

Learning and innovation are crucial aspect of human development. Direct transaction of technology and indirect spill-over through various channels such as copying, reverse engineering and movement of workers and managers between foreign and domestic companies also facilitate transmission of knowledge to the rest of the economy. Linkages between SEZ and Social development are provided in Fig 1**.**

The above analysis suggests that SEZ impact on social development three channels: employment generation, skill formation and technology up-gradation.

The SEZ (2007) activity brings out beneficial impacts rather than adverse impacts in nature. Some adverse impacts of the SEZ activity on social development SEZ activity may increase pollution level and social security problems may in the region due to

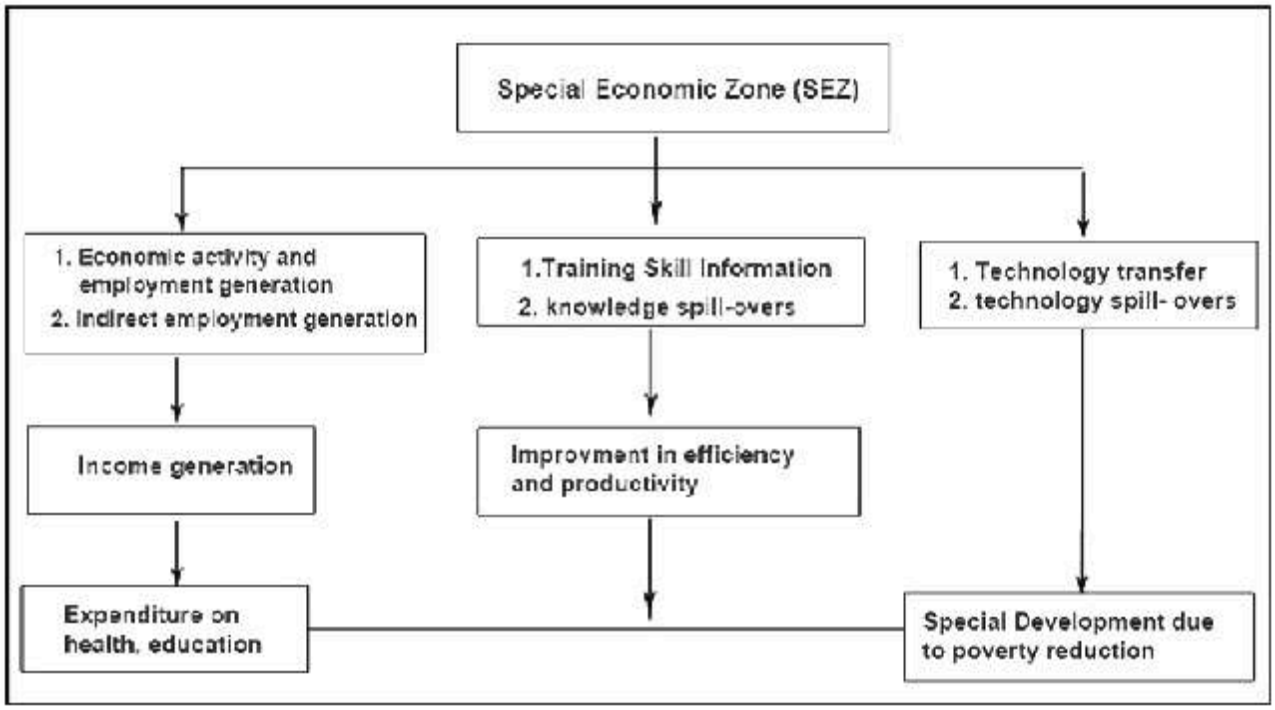

Fig. 1: Linkages between SEZ and Social evelopment

influx of population. Dust pollution due to transportation in dry areas. Change in population density and diversity through the immigrants may cause cultural and health related problems in the region.

Mitigation measures for impacts due to SEZ

With the growth of SEZ activity there is also growth of various other similar activities large scale of impacts are experienced in the past one of them is influx of population in the SEZ area. Hence the SEZ activity requires much different treatment so far as planning SEZ impacts concerned.

Affected population should get maximum benefit and adequate facilities, like health care, education, recreation and employment etc. People residing in near by SEZ areas must be provided safe drinking water supply, sanitary system and other essential amenities. Adequate space must be given for recreation activities and parks. Tree plantation shall be undertaken to keep the environment clean and safe. Appropriate accompaniment must be offered for economic reintegration of the affected population, through educational and vocational training. Workers working in high noise environment in SEZ activity should be provided protective devices like acoustic wool, earplug, earmuffs etc. Workers working in dust screening filters should be provided air filter masks to prevent from dust pollution. The social and economic consequences of SEZ provided employment and increasing the income of local population. SEZ have the potential for generating direct and indirect impacts and that can be used as an effective policy instrument in promoting employment and human capital and alleviating poverty. Proactive government polices May therefore play a crucial role in this regards. The government intervention may be engaged at various levels like labour standards, conditions of work, health and safety standards and the creation of support infrastructure for education and training of labour, SEZ can be used as a policy instrument in upgrading skills and building of human capital.

SEZ areas generally focused the quality of life of the people in the region and with respect to positive and negative Impact of SEZ activity. The elaborative SEZ Impact studies with social environment as a vital part reflected the various benefits to the local population by the SEZ activities both economically as well as the infrastructure development of the area:

i. The SEZ areas are benefited by the welfare activities carried out by SEZ authority such as provision of health care centre, education

institution, water supply and electricity etc.

ii. In some region certain auxiliary industries have established which has increased the employment opportunities for the local people.

iii. SEZ areas showing quality of life (QoL) are satisfactory level

iv. The negative impacts reported from increase pollution level

Employment generation both direct and indirect is a most important channel; through SEZs have impact on social development and poverty reduction in India. Hence employment generation potential of SEZ is rather large, they have contributed significantly to employment generation at the regional level. There is a wide consensus of the central role of employment in poverty reduction. SEZ can be use as an effective policy instrument in alliterating poverty. Poverty reduction thus calls for the creation of remunerative regular and good quality jobs in the labour market. Working conditions in the SEZ in terms of social security benefits, transport facilities, health facilities and working environment are also better than those in the same types of jobs in the rest of the economy. This has had a direct effect on the standard of living of workers in SEZ.

The role of SEZ in human capital formation appears to be rather limited. SEZ contributed as an engine for promoting new knowledge, technologies and innovations through technology transfers and technology creation.

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Galactogogue Effect of Garden Cress Seeds on Lactating Rats

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Abstract : To find out the galactogogue effect of garden cress seeds *(Lepidium sativum Linn)* 30 healthy lactating rats were equally divided into six groups, three kept as control for their respective treated groups and were given stock diet only. Whereas, other three groups were maintained on 20% seed powder mixed with stock diet for 7,14 and 21 days during their lactation period. The day next to parturition was taken as the first day of lactation. The seeds showed enhanced milk producing effect which was evident from increased mammary gland weight, histological and biochemical findings as well as increased number and size of alveoli, secretory materials and higher level of serum prolactin as compared to those of control groups. The maximum effect was noted in the 14 days treated group, that is, during the mid lactation period.

Key words : Mammary Gland, Secretory Material,Prolactin

Introduction

Garden cress seed *(Lepidium sativum Linn)* is well known for its medicinal as well as nutritional properties (Gopalan *et al., 2000).* Some work has been done on the nutritional effect of the seed on human and experimental animals (Datta and Ghosh, 1990; Datta and Ghosh, 1992). However, it was interesting to note that the seeds possess galoctogogue properties (CSIR, 1962). An extensive review of literature revealed no experimental evidence to support its galactogogue either in human or in laboratory animals. Therefore, present study is an attempt to investigate the galactogogue effect of garden cress seeds on laboratory rats at their lactating period after administration through diet at a level which would be safe for different experimental procedure (Loomis, 1978; Sarkar, 2004).

Materials and Methods

The study was carried out on young female virgin albino rats (200-220g) of Ducray Strain obtained from local animal dealer. The experimental animals were acclimatized in the laboratory condition for 7 days. All the animals were handled with utmost humane care.

The female rats at their estrus were kept with male rats in the ratio 2:1. After successful mating, all the pregnant rats were kept isolated and continued feeding stock diet and tap water *ad libitum* till the end of the gestational period. After delivery each rat was kept along with their young ones (pups) on carefully made grass beds. Seven to ten pups were produced by each rat.

Experiments were carried out on 30 healthy female lactating rats equally divided into six groups. The treated groups were given experimental diet for 7 days (Group I), 14 days (Group II) and 21 days (Group III). Each group had its own control group reared on stock diet only. The treated groups were given garden cress seed powder and stock diet mixed in the ratio of 1:4. That is the seed mixed at 20% level did not differ in taste or palatability from the control diet and had no adverse effect (Loomis, 1978; Sarkar, 2004)

Throughout the experiment total food intake per rat of both control and treated groups was recorded. Body weight was recorded at the end of the experimental period of 7 days for Group I, 14 days for Group II and 21 days for Group III of both control and treated rats.

Animals were sacrificed with an overdose of ether anaesthesia after 12 hours of fasting. Blood was collected for estimation of haemoglobin level by cyanomethaemoglobin method (Dacie and Lewis, 1968). Serum was collected for prolactin estimation by Liqui- Prolactin enzyme immunoassay test kit (Jeffe and Behrman, 1979) and weight of mammary glands of both control and treated groups was recorded. Tissues were kept into Bouin's fixative for routine histological procedure (Raghuramulu *et al.,* 1983). Paraffin sections (7 µm) were stained with Haematoxylin-Eosin and Masson's Trichrome stain for microscopic examination.

Results

The statistically significant gains of body weight

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of the treated lactating rats in Groups I, II and III (T1, T2,T3) occurred than those of corresponding control lactating groups (C1,C2, and C3). The body weight gain is maximum in Group II that is the 14 days treated group (Table 1).

Anatomical examination of mammary glands showed an outward manifestation in glandular sizes which too was greater than that of the corresponding control. The abdominal mammary glands were more prominent.

Presence of secretory material was more precipitated in Group T2. Group T3 apparently did not show much difference from Group C3. The size and number of alveoli had increased particularly in Group T1 and T2. The hypertrophy of the alveoli was more pronounced in Group T2 as compared to those of Group T1. The maximum efficacy was found in Group II of experimental animals on their 14th day of lactation. In Group I and Group II, the alveoli showed the accumulation of secretory material. In Group II, where rats were fed, garden cress seeds for 14 days showed maximum enlargement of alveoli and more accumulation of secretory materials (photos. 2 and 4).

Discussion

Increase in weights of mammary glands and serum prolactin level (Table 1) corresponds to the histological findings. The maximum increase in weight was found among rats of Group II (14 days treated rats), showing an augmented proliferation of alveoli and enlargement of the alveolar cavity sac as compared to those of Group I and Group III. The weight of the mammary gland was found to the highest in Group II, which is in conformity with the microscopical structure (Photos: 1-6).

Histological observation of the mammary glands Indicate that there was an enhanced activity in the treated groups (T1,T2 and T3) as compared to control groups (C1 C2 and C3). Therefore, it was evident from the histological observation that the mammary gland of the treated groups was conspicuously hyperactive in contrast to those of non-treated lactating animals. A decrease in the thickness of connective tissues surrounding the alveoli has also been observed indicating thereby an increased proliferation of the alveoli. This had one-to-one correspondence with the increase in weight of the mammary gland (Table 1).

The garden cress seeds enhanced the haemoglobin (Hb) level of blood of treated rats of 7 days (Group I) 14 days (Group II) and 21 days (Group III), satistically as 99 per cent level of significance over the untreated (Control) lactating rats (Table 1). Present findings confirm similar propensity for incremental effect of garden cress seeds on hemologlobin level of young women and adult female rats already reported (Dutta and Ghosh 1990; Datta and Ghosh 1992).

The present findings on the galcotogogue effects of garden cress seeds are evident from the enhanced development of mammary gland i.e. weight increase of mammary gland, proliferation of alveoli and increased accumulation of secretory material in treated lactating rats compared to their corresponding control.

Table 1 : Experimental findings of control and treated lactating rats of Group I (7 days), Group II (14 days) and Group III (21 days)

Parameters		Group-I		Group-II		Group-III	
		Control	Treated	Control	Treated	Control	Treated
		(C1)	(T1)	(C2)	(T2)	(C3)	(T3)
Body weight (g)	Initial	204.6 ± 6.03	202.0±3.07	213.6±8.19	209.4±3.56	203.0±1.22	202.0±1.0
	Final	210.6± 6.07 N.S.	210.0± 4.64 N.S.	222± 9.23 N.S.	227.0± 8.98 N.S.	206.0± 0.70 N.S.	210.8± 2.74[a]
Mammary gland weight (mg)		27.7± 0.6	84.8± 1.57[c]	40.4±1.54	114.6±2.18[c]	29.0± 0.84	83.6± 1.31[c]
Prolactin level (ng/ml)		22.6±0.84	68.60±1.04[c]	24.6±1.61	76.0±1.81[c]	-	-
Hemoglobin level (g/dl)		10.1±0.16	12.76±0.55[b]	10.89±0.38	12.59±0.26[b]	10.59±0.27	13.39±0.44[c]

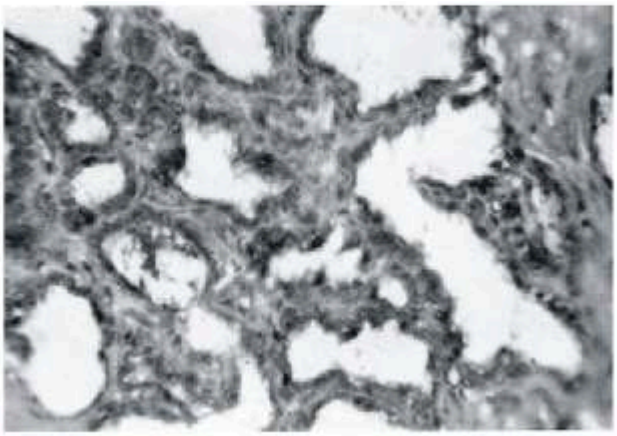

Fig. 1:Active mammary gland of control rat on 7th day of lactation Alveoli are seen lined by a thin layer of epithelial cells. Connective tissue surrounds the alveoli : x 275

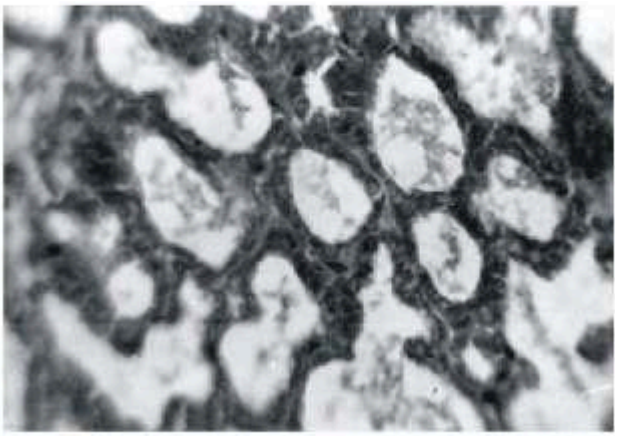

Fig. 2: Mammary gland of treated rat on 7th day of lactation. Alveoli are larger in size with secretory materials compared to control and surrounded by loose connective tissue : x 275

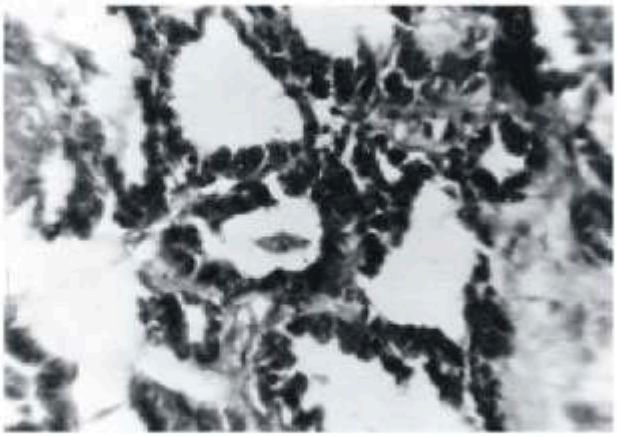

Fig. 3: Lactating mammary gland of 14th day control rat. Alveoli are separated by highly cellular connective tissue : x 275

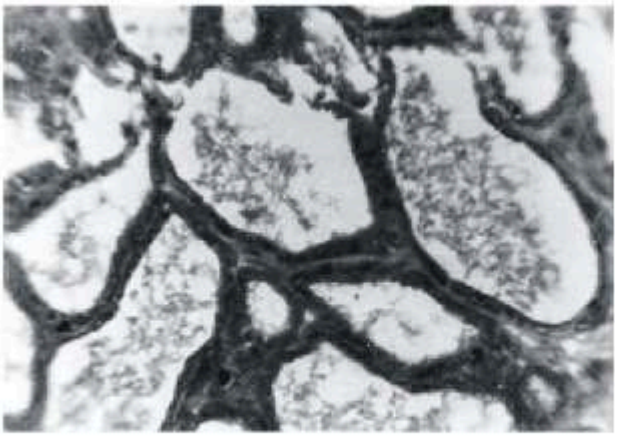

Fig. 4:Treated mammary gland of 14th day rat showing larger alveoli with secretory material. Connective tissue area is reduced. Inter-lobular septum is also visible : x 275

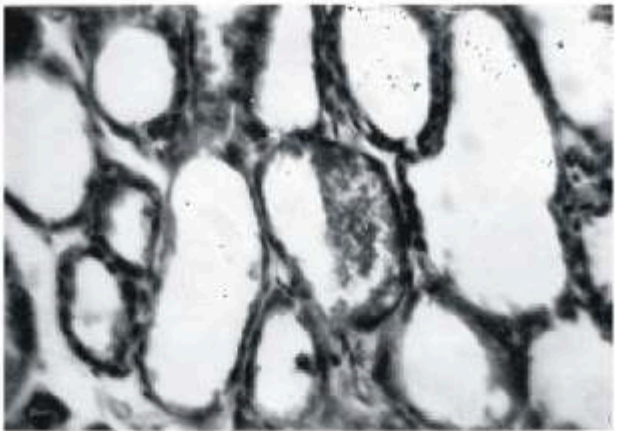

Fig. 5: Mammary gland from control rat on 21st day of lactation with irregular alveoli and reduced surrounding compared connective tissue to 7th and 14th day control : x 275

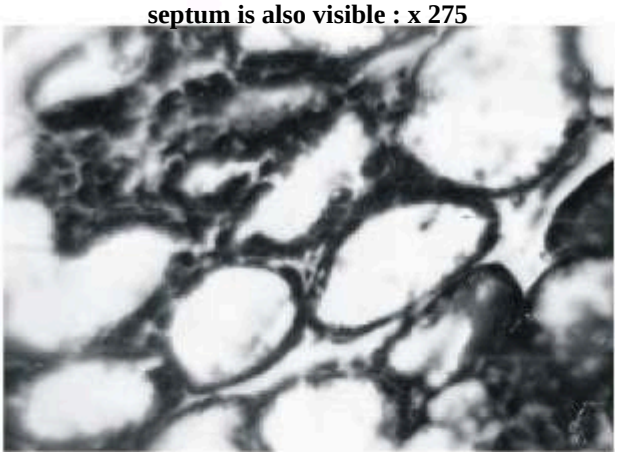

Fig. 6: The alveoli size of 21st day treated lactating rats are almost same as 21st day of control rat. The surrounded connective tissue has increased compared to 14th day treated lactating rats : X 275

So it may be concluded that the seeds, if used during the early phase of lactation can produce a desirable effect. Further, it may be added that the high iron content of the seed (100 mg/100 g) (Gopalan *et al*., 2002; Datta and Ghosh, 1992) can improve nutritional status and rectify anaemia usually prevalent among postpartum women in our country (UNCIEF, 2000).

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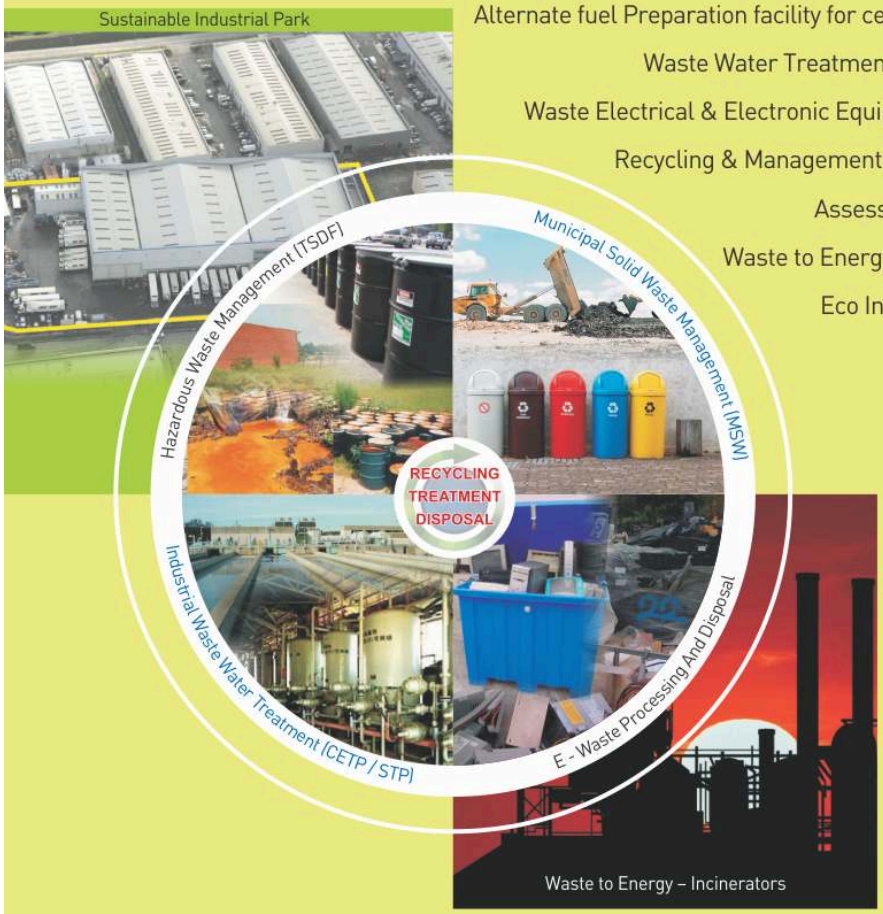

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