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SOCIAL AND CONSERVATION VALUES OF TWO SACRED GROVES OF BANKURA DISTRICT, WEST BENGAL

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Abstract ■ *This paper attempts to study how socio-cultural and conservation ethics of tribals of Bankura district in West Bengal preserve plant diversity in two sacred groves. The study documents that in addition to conserving forty (40) angiospermic taxa having social and economic values, the groves provide cultural space for community festival, new alliance and fraternity. The present findings demand similar interdisciplinary initiative to study more sacred groves and also call for the protection of the groves.*

Introduction

Sacred groves are parts of forests and or tree groves protected and managed by local people on socio-cultural grounds. They are considered as abodes of deities, spirits or supernatural powers. This phenomenon of tree and forest preservation is distinctly different from the propitiation and protection of individual and scattered trees/plants like *Aswatha*, *Bat*, *Bel*, *Tulsi* etc. Sacred groves are found in Africa, America, Asia, Australia and Europe. In India, they are reported to be found in Andhra Pradesh, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Meghalaya and tribal dominated areas of Jharkhand, Orissa and West Bengal. These groves, although known variously by local names, share common characteristics in protecting biodiversity and providing cultural space for the local people. (Gadgil and Vartak, 1975; Bhakat 2003, 2009, Malhotra et al., 2007, Kisku et al., 2010) With this background, this study attempts to highlight the socio-cultural roles and conservation values of two (2) isolated sacred groves located a few kilometers apart in Sarenga police station areas of Bankura district in West Bengal. These two sacred groves are situated on public lands along the border area of Bankura-Midnapore (W) districts and are maintained mainly by village tribals.

Study Area

Bankura (22°36′ – 23°38′ N Latitude and 86°36′ – 87°46′ E Longitude), one of the southern-most districts in West Bengal, covers an area of 6,882 km^2. The district is bounded by Burdwan district on the North and North-East, Hooghly district on the South-East, Midnapore(W) district on South and Purulia district on the West. It is drained by Damodar,

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Dwarkeshwar, Kanswabati and Silabati rivers and their tributaries. Geomorphologically, the area is marked out into three (3) distinct natural zones: 1. The plain-land at the East, 2. The hilly areas to the West and 3. A connecting undulating tract in the middle. The strategic position of the district between the plateau of Chotanagpur and the plains of lower Bengal forms a continuum, facilitating plant and human migration. On account of its unique locational uniqueness, a large portion of the district is covered with pre-dominant dry-deciduous forests with regenerating *Sal* plantations. Since the area comes under the middle tribal zone of India, it supports a substantial populations of different ethnic groups like *Bedia*, *Koramudi*, *Mahalis*, *Santhals* etc. who reside in the forest-fringe villages. These people have distinct socio-cultural attachments with the surrounding plants and forests. They not only depend on the forest resources, but also protect and conserve plant resources in the form of sacred groves.

Methodology

In the course of investigation for one year (2009-2010), the present sacred groves and their adjoining villages were surveyed during various seasons. Identification of plants was done on the "spot-identification" method. Unknown plants were identified through relevant literature (Prain, 1903; Sanyal, 1994) following the standard method. While enumerating plants, species name is arranged alphabetically followed by habit, local name, use(s) and number of individuals of a species growing in the sacred grove(s). Social and economic values of plants and cultural aspect of sacred groves were studied through participatory method during which local villagers were interviewed and cross-interviewed. Oral history from elder generation was collected to document the change that has taken place during the recent period. The results thus generated were verified with the relevant available literature. (Shiva, 1998; Dhiman, 2003; Paria, 2005).

The Sacred Groves

The Karnbhanga sacred grove and the Gobindapur sacred grove, each locally known as *Baram than* (altar of a local female folk deity *Baram*), are located along the south-north bound Pingboni (West Midnapore district) – Sarenga (Bankura district) metallic road on the north-west and south-east outskirts of respective village of the same name. While the former represents a 250-yr old forest patch spread over a 5000-m^2 public land, the latter is located on a 4000-m^2 forest land and is assumed to be of approximately 350 years old. Both the groves support few old trees, shrubs, herbs and climbers and house the local folk deity *Baram*

Table 1. Profile of the Studied Sacred Groves

Name, Age (years) size (m^2)	Deity	Community Protecting	Adjoining villages / town	Ownership status	Dominant tree species
Karnbhanga, 250,5000	Baram	Lohar, Mahato, Santal	Hatuadi, Gopaldanga/ Sarenga	Public Land	*Alangium salvifolium, Borassus flabellifer, Acacia auriculiformis, Ficus sps., Shorea robusta, Terminalia arjuna*
Gobindapur, 350, 4000	Baram	Mahato, Santhal	Gobindapur, Karabara/Sarenga	Public Land	*Adina cordifolia, Aegle marmelos, Alangium salvifolium, Morinda aungustifolia, Streblus asper, Eucalyptus sp.*

(Table 1). The goddess is aniconic, represented by stones smeared with vermillion lying under old trees. The deity is often presented with votive offerings of burnt clay idols of horses and elephants. She is believed to be of ferocious in nature and often demands animal (hen, goat etc.) sacrifices. During the annual *paus sankranti* – a ritual celebrated on the last day of Bengali month *Paus* (middle of January) – local people mainly, Santals, Mahatas, Lohars etc. gather inside the groves and worship the deity. Additionally, the goddess is propitiated in Gobindapur sacred grove only during *Chaitra Sankranti* (middle of April) annually. Moreover, the deities may be worshipped on Tuesday or Saturday or any day deemed to be auspicious by the local people. It is believed that the feminity of the deity is indicative of the cult's origin in the hunting-gathering stage when the human society was not yet settled in permanent dwelling (Kosambi, 1962). This assumption can be supported by the fact the place of worship (tree grove) is away from the village. Since the groves are abodes of deity, cutting or even lifting of any plant from the groves is totally forbidden. And these social norms and taboos surrounding the groves promote their protection and conservation.

Socio-Cultural Importance

All sacred groves, irrespective of their size and origin, are institutions of socio-cultural practices. And this is true for the present sacred groves also. The folklore goes that worshipping the deities begets well-being and prosperity of the villagers. The grove also provides cultural space to the communities as a common property resource. It acts as a platform for assertion of group identity and group solidarity. The local people form new alliances while participating in the different sacred grove-based rituals and festivals. Propitiation of deities inside the grove has economic relevance in terms of good rainfall, prosperous agricultural production, welfare of domestic animals and source of medicine, timber, fruits etc. during scarcity. Moreover, the groves provide moral support and guidance to the communities.

Conservation Value

The present study on Karnbhanga and Gobindapur sacred groves reveals that owing to high-level of protection offered on socio-cultural grounds, they provide optimum conditions congenial for plant growth and survival. While Karnbhanga SG bears twenty-two (22) species of angiosperms, Gobindapur SG supports twenty-eight (28) species. In a combined way, these two groves house forty (40) species of angiosperms covering herbs (13 spp.), shrubs (6 spp.), trees (17 spp.) and climbers (4 spp.), of which ten (10) species are common to both (Table 2). Among the total species, 6 bear edible part(s), 3 are used as fire-wood, 1 is garden plant, 26 have medicinal value, 8 are sacred species, 6 have timber value, 1 is used as tobacco-wrapping plant, 2 are used for plate-making purpose and 1 is used to prepare mat. The local people do not harvest any plant or plant part since the sacred groves are considered as abodes of deities, but may collect plants after social sanction from the custodians of the groves, and that too in a sustainable way. As such the groves serve as genetic resource and seed bank for future silvicultural studies. The old tree species of the grove indicate the composition of the erstwhile forest flora of the region.

Threats

The sacred groves though fairly well-protected by the villagers, are under mild threats due to agricultural encroachment, exotic weed invasion and erosion of people's moral values towards plants and forests.

Table 2. Plants of Karnbhanga (K) and Gobindapur (G) sacred groves (H – Herb, S – Shrub, T – Tree, C – Climber; E – Edible, F – Fire-wood, G – Garden Plant, M – Medicinal, S – Sacred, Tim= Timber

	Species	Habit	Local Name	Value(s)	Sacred Grove(s)
1.	Acacia auriculiformis	T	Sonajhuri	F, Tim	K15
2.	Achyranthes aspera	S	Apang	M	G Many
3.	Adina cordifolia	T	Haldu	S, Tim	G5
4.	Aerva lanta	H	Chaldhowa	M	G Many
5.	Ageratum conyzoides	H	–	M	G Many
6.	Alangium salvifolium	T	Akar	E, F, Tim	K4, G2
7.	Andrographis paniculata	H	Kalmegh	M	G Many
8.	Borassus flabellifer	T	Tal	E, Tim	K4
9.	Calotropis gigantea	S	Akanda	M, S	K5
10.	Chrysopogon aciculatus	H	Chorkanta	–	G Many
11.	Curculigo orchioides	H	Talmuli	M	K Many
12.	Diospyros melanoxylon	T	Kend	E, Tobacco, Wrapping	K4
13.	Eclipta alba	H	Keshraj	M	K Many, G Many
14.	Eucalyptus globulus	T	Eucalyptus	M, Tim	G20
15.	Ficus glomerata	T	Dumur	E	K2
16.	F. religiosa	T	Aswatha	S	K2
17.	Gymnema sylvestre	C	Gurmar	M	K5, G4
18.	Hemidesmus indicus	C	Anantamul	M	G Many
19.	Holarrhena pubescens	T	Kurchi	M	G4
20.	Hygrophilla schulli	S	Kulekhara	M	K Few
21.	Phoenix sylvestris	T	Khejur	E, Mat-making	K1, G4
22.	Poenix acaulis	S	Bankhejur	Mat-making	K2
23.	Rauvolfia tetraphylla	S	Bansarpagandha	M	G Few
24.	Shorea robusta	T	Sal	S, Tim, plate-making	K2
25.	Sterblus asper	T	Shorea	S	K2, G3
26.	Strychnos nux-vomica	T	Kuchila	M	G1
27.	Ventilago denticulate	C	Raktapita	M	G1
28.	Terminalia arjuna	T	Arjun	M	K2
29.	Thevetia peruviana	T	Kalke	G, S	G2
30.	Aegle marmelos	T	Bel	E, S	G5
31.	Morinda aurgustifolia	T	Ach	M	G2
32.	Tridax procumbens	H	Tridaksha	M	K-Few, G. Many
33.	Ocimum sanctum	H	Tulsi	M, S	G Many
34.	Cyperus rotundus	H	Mutha	M	G Many
35.	Commelina benghalensis	H	Nilghas	M	G Many
36.	Portula guadrifida	H	Bangima	M	K Many, G Many
37.	Boerhaavia repens	H	Punarnava	M	K Many, G Many
38.	Sida cordifolia	H	Berela	M	K Mang
39.	Tinospora cordifolia	C	Gulancha	M	K Few, G Few
40.	Lantana camara	S	Putus	F, M	K Few, G Few

Conclusion

It has been found that the studied sacred groves, in addition to protecting agood number of plant species through the traditional *in sifu* method, also provide social spaces wherein the local people exchange their cultural views and make new alliances during various grove-based rituals and festivals. The studied sacred groves are comparable to others in West Bengal and India. Thus, there is an urgent need to conserve the grove and also to study similarother groves which act as islands of biodiversity. And for this, awareness should be initiated among the stakeholders, local villagers and concerned individuals about the importance and relevance of conserving sacred groves.

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Application of "Participatory Rural Appraisal" technique for critical analysis of micro-farming situations in traditional pond fishery of a selected village in West Midnapore district

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Abstract ■ A team of scientists from the Universities analyzed the micro-farming situations in traditional pond fishery of Barua village in West Midnapore district of West Bengal state of India using "Participatory Rural Appraisal (PRA)" technique. The village has a population of over 5000 with 765 households out of which 95 households have fishponds. Different PRA tools like group discussion, key informant's interview, direct observation and diagramming were employed for analysis. Available resources, farming practices and constraints in the traditional pond fishery of the village were identified during the study. Analysis of problem-cause diagram revealed that unscientific stocking density, improper species combination, lack of integration with livestock, non-inclusion of high-value species like prawn, non-use of supplementary feed etc. are the important reasons for low yield of fish and low profitability from fish farming system in the locality. To tackle above problems, four numbers of technological interventions were designed with farmer's participation and implemented in farmers' ponds either in form of verification trial or on-farm Trial. Optimum stocking of fingerling @ 8000/ha. increased the fish production by 57% in the village with average yield of 2506 kg/ha/crop. Integration of duck @ 250 / ha. with fishery, increased the farm profitability by 125% with fish production of 3596 kg/ha./crop. By using supplementary feed of rice bran and oil cake (1:1), higher fish production of 3332 kg/ha./crop was obtained. Inclusion of *M. rosenbergii* in carp farming system resulted in 92% increase in farm income. During the study 8 numbers of Indigenous Technical Knowledge (ITK) were also documented from the local fish farmers of the study area.

Key words: Participatory Rural Appraisal (PRA), micro-farming situation, pond fishery

Introduction

In India, the agricultural research and technology are supported by five agro-ecosystems, *viz.* irrigated, rainfed, coastal, arid, and hill & mountainous agro-ecosystems. Assessment of a technology under various micro-farming situations is essential in order to realize the fruit of that technology by the farmers living in different agro-ecosystem. Unfortunately in India, research and extension

programmes often follow "Top down" approaches. These approaches, however, do not address the constraints and development potentials of the farm house-hold systems and rural communities in a comprehensive way. Participatory Rural Appraisal (PRA) technique has been developed to overcome these shortcomings and it is the reversal from "Top down" to "Bottom up" process. Site-specific assessment and refinement of technologies could be made through farmer's participatory research and such technologies made be accepted by the farmers (Johnkutty *et al.*, 2001) In aquaculture sector, the socio-economic conditions of traditional fish farmers in India have not improved to the desirable extent, because the existing research and extension set up has failed to meet the needs and aspiration of farmers. In this context one of the important investigations has made by Mandal *et. al* in 2008a. Many innovations being proposed were not being adopted by farmers (Mukhopadhyay, 1999). The major reason for this shortcoming is that researchers and development planners do not have proper perspective of the resources, environment, problems and needs of poor farmers. Proper matching of farmers' problems with possible technological interventions is necessary to enhance technology adoption (Anantaraman *et al.*, 2001).

Participatory Rural Appraisal is a technique to analyse the micro-farming situations in a particular area by providing sufficient background information on the land and its people. It includes the critical analysis of available resources, infrastructure facilities, employment and income generation activities, problems and possible remedies *etc.* (Goswami *et al.*, 2002). The present study employs "Participatory Rural Appraisal" technique for critical analysis of micro-farming situations in the traditional pond fishery of a selected village in West Midnapore district of West Bengal.

Materials and Methods

A team of scientists from the Universities analyzed the micro-farming situations in traditional pond fishery of Barua village in West Midnapore district of West Bengal state of India using "Participatory Rural Appraisal (PRA)" technique. The Barua village is located at latitude of 22°25' N and longitude of 87°19' E. The village has a population of over 5000 with 765 households out of which 95 households have fishponds. Though the micro-farming situations were analysed for agriculture, livestock and fishery resources of the village, only information related to fishery resource are discussed in the present paper. A cross section of people including elders, practicing farmers, youth and women of the village took part in PRA. PRA tools as suggested by Conway *et al.* (1987) were followed in study. The sequential steps followed in PRA were: preliminary visit ? rapport building ? team formation ? exploration of secondary data ? data collection ? data analysis ? implementation of action plan through technical intervensions ? monitoring and evaluation ? feed back on success/failure of interventions ? planning for extrapolation/ refinement. Additional tools, as proposed by Chand and Goswami (2004), were employed for analysis and it included group discussion, key informant's interview, direct observation and diagramming.

Based on the micro-farming situations, available resources, farming practices and constraints were identified. The project team implemented four numbers of fishery related interventions with farmer's participation. These interventions were designed to solve the problems faced by the fish farmers and implemented either in form of verification trial or on-farm trial. The progress of the interventions was constantly monitored through regular farm visit, collection of field data, conducting farmers' meet etc.

Table 1: Details of tools / methods used in agro-ecosystem analysis

Events	Tools/methods used	Remarks
Identification of village	Participatory discussion with district government officials of fishery department and rural development	5 villages having good numbers of fish ponds were short listed and ultimately Barua village was selected.
Understanding geographical layouts, micro-farming situation, farming practice, etc. and preparation of transect map	Transect walk, mapping and modeling	Mapping with sketch pen on paper, modeling on the floor (by farmers)
Historical background of village, changes and trends, farming practices, etc.	Time line	Participatory group discussion with elderly people
Preparation of village map, land use map, mobility map, rainfall pattern, etc.	Mapping and modeling	Mapping with sketch pen on paper, modeling on the floor (by farmers)
Trend and changes in the village	Mapping and modeling	Mapping with sketch pen on paper, modeling on the floor with twigs, seeds etc.
Preference of species / practices in fishery	Matrix scoring and ranking	Assigning scores on black boa rd with chalk
Resource flow analysis of inputs and outputs	Resource flow mapping	Mapping with sketch pen on paper
Institutional linkage of the village	Venn diagram	Using paper cut out circles
Income-expenditure break up	Livelihood analysis	Pie diagram using chalk, colour powder
Problem identification	Matrix ranking, focused group interview	Ranking by farmers
Problem cause relationship	Problem cause diagram	Diagram by farmers
Intervention identification, nature and details of intervention	Participatory group discussion and focused group interview	Farmers decided the intervention and core scientific team facilitated their involvement
Documentation of Indigenous Technical Knowledge (ITK)	Group discussion	Farmers disclosed ITK

Table 2: Details of technological interventions used in the study

Intervention No.	Name of Intervention	Details about intervention
Intervention 1	Optimizations of stocking density of Indian Major Carp in composite fish farming	**Control**: Excessive stocking of fish fingerling in the pond
		Treatment: Optimum stocking of fingerling @ 8000/ha.
Intervention 2	Integrating duck rearing with fish farming	**Control**: Traditional fish farming without integrating livestock
		Treatment: Integrating duck @ 250 /ha with fish farming
Intervention 3	Supplementary feeding of rice bran and oil cake in 1:1 ratio through feeding bags	**Control**: No supplementary feed in fish farming
		Treatment: Supplementary feeding of rice bran and mustard oil cake in 1:1 ratio through feeding bags in carp farming
Intervention 4	Inclusion of high value item – freshwater prawn in carp farming system	**Control**: Traditional fish farming with Indian Major carp only.
		Treatment: Culture of freshwater prawn, *Macrobrachium rosenbergii* along with IMC.

Results and Discussion

Village map: Social map of Barua village drawn by the villagers gave a bird's eye view of the village in totality. It gave a fairly good idea about the settlement pattern of houses, availability of infrastructure facilities, land use pattern, religious and social institutions located in the village. There were 765 houses in the village and the settlement pattern was of typical *Bengali* type with clusters of houses scattered throughout the village.

Soil map of the village showed that the village had three types of soil, *e.g.* clayey, sandy and loamy out of which the area under clayey soil is more. Hydrology map of the village depicted that the village had 18 big size ponds (>1.0 ha.), 27 medium size ponds (0.4 to 1.0 ha.) and 50 small size ponds (< 0.4 ha.). Fish farmers of the village mainly culture Indian major carps in their ponds.

exercise consists of a combination of outsides and local farmers. The village transect of the Barua village represented the cross section of the village indicating topography, soil type, crops grown, livestock details, fishery resource, irrigation source etc.

Time line: The important events occurred in the village are depicted below in form of time line.

Trends and changes:

Information on trends and changes of last 30 years in Barua village were collected from the elderly villagers and depicted with the help of various symbols at fig. 1. This figure only shows the trend and not the proportionate numbers. The population of the village has steadily increased and has nearly doubled in last 40 years. Number of houses has also increased proportionately. The literacy percentage among the villagers has shown increasing trend. The

Table 3: Time line of Barua Village

Year	Event
1962	Construction of road to Midnapore town
1965	Occurrence of drought
1966	Introduction of radio
1976	Government primary school
1980	Implementation of land reform act
1981	Installation of deep tube well & introduction of HYV paddy
1986	Introduction of composite fish culture involving three species of IMC
1988	Outbreak of Epizootic Ulcerative Syndrome (EUS) disease in fish
1990	Arrival of electricity
1997	Arrival of cable TV
2000	Opening of English medium school

Village transect: Transect is an observatory walk or trek across the country side in a given village. Through transects, one gets insights and introduction into the nature and complexity of farming, resource conservation and management which conventional approaches do not usually offer. The group doing the transect

area under fish farming has increased marginally over the years, whereas fish production has increased significantly after 1985, *i.e.* with the introduction of composite fish culture technology. Popularity of supplementary feed in fish farming has developed recently among the farmers and catching up slowly.

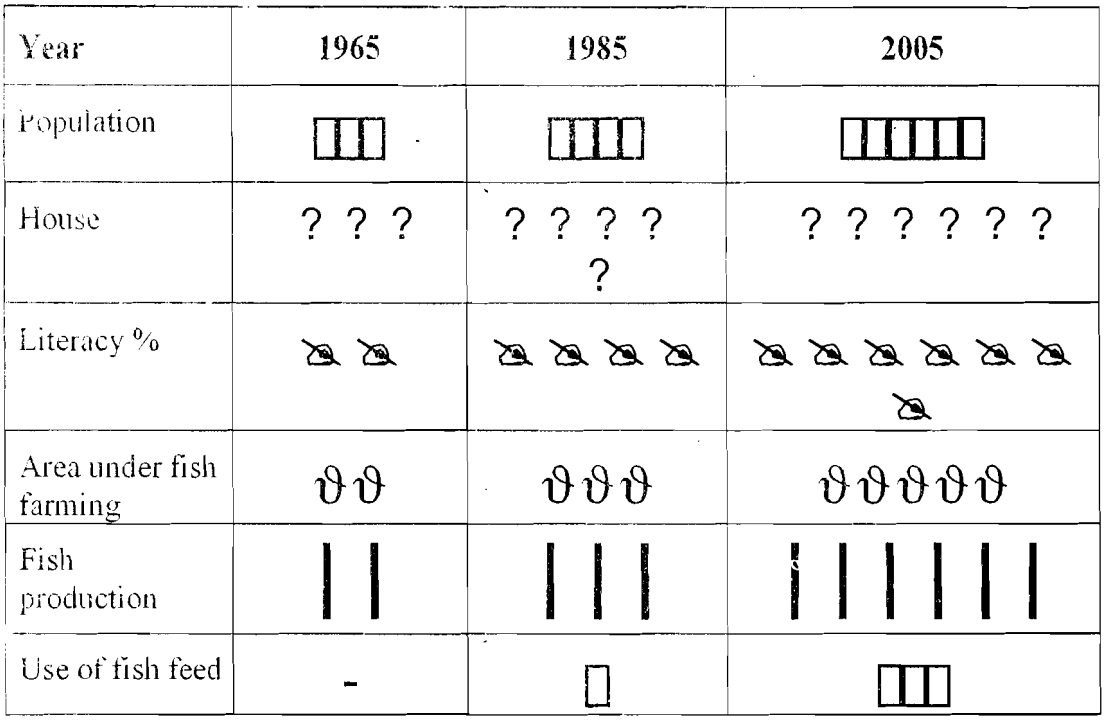

Fig. 1: Trends and changes in Barua village (through pictorial depiction)

Seasonality: Seasonality is a method to study the variation of different farming parameters according to seasons. The Barua village receives maximum rainfall during June to September and it tapers off in October. Farmers generally stock fish fingerlings during August/ September when ponds are full with rain water. The incidence of disease in fish is common during pre-summer and summer months. Diseases like dropsy, fin-and-tail rot, *argulus* attack are common in carps. Labour requirement in village is more during the months of June, July, August, November and December (i.e. for agriculture and fishery).

Resource flow of the village: The villagers of Barua depend on the outside areas for meeting their resource need to some extent. Inflow items like fish feed, lime, fertilizer, chemicals, fish seed etc. are procured by the farmers from near by markets. Fish is sent from the village to local market after meeting the demand of the village. Manpower required for all types of fishery activities is met by the villagers themselves. Moreover, during lean season excess manpower goes to nearby town in search of work.

Mobility: Barua village has primary school (two), sub-health center, Gram Panchayat office (local self-govt.), temples (two) and play grounds (three). Market, post office, high school, bank and fishery office are located at 2 to 3 km a way from the village.

Livelihood analysis: Livelihood analysis is carried out to understand the income and expenditure pattern of the farm families. Entire farm families are divided in to three categories based on their monthly income (*i.e.* rich: > Rs. 5000 p.m., medium: Rs. 1500-5000 p.m. and poor: < Rs. 1500 p.m.). In Barua village, 5% of total families were rich, whereas 35% families belonged to medium and rest 60% came under poor category. The livelihood

analysis was carried out for each category separately by interacting with the representative of the categories.

Decision analysis: Decision analysis plays an important role at all levels in the hierarchy of agro-ecosystem. Farmers' choice in selection of fish species, management decisions like how to culture, where to procure inputs, etc. are the numerous decisions a farm family normally takes. Gender also plays an important role in this. Gender involvement in decision making analysis at Barua village is given at table 4.

Documentation of Indigenous Technical Knowledge (ITK): ITK is the sum total of knowledge and practices, which is based on people's accumulated experiences in dealing with situations and problems in various aspects of life. ITK is the total knowledge that is unique to the given culture or society. Such knowledge is gained over a period of time and passes on from generation to generation by word of mouth. During the project period, the scientists could identify the following 8 numbers of ITKs from the local fish farmers.

Table 4: Gender involvement in decision making and implementation

Issues	Who initiates	Who participates	Who decides	Who implements
Type of fish culture	Man	Both	Man	Man
Purchase of fish seed	Both	Both	Man	Man
Purchase of fish feed	Both	Both	Man	Man
Pond management	Both	Both	Both	Both
Domestic activities	Woman	Woman	Woman	Woman
Money management	Both	Both	Man	Man
Child education	Both	Both	Man	Man
Social commitment	Both	Both	Both	Both

Table 5: List of ITKs documented from the study area

S.N.	Name of the fishery based ITK
1	Application of banana pseudostem in fishpond to improve water quality and check fish mortality
2	Broadcasting of ash (of freshly burnt paddy straw) in fishpond to check disease outbreak in fish
3	Use of earth worm / roasted pupa for angling fish
4	Use of mixture of lime and neem leaf powder (2:1 ratio) in ponds to control disease in fish and prawn
5	Application of mixture of neem oil and turmeric powder on wounds of fish affected by Epizootic Ulcerative Syndrome (EUS)
6	Use of roasted rice husk mixed with cow dung and kerosene as attractant in catching fish and prawn
7	Use of tree branches as fish / prawn aggregation device in ponds
8	Use of special traps made up of bamboo strips to catch miscellaneous fish from paddy fields

Micro-farming situation identified: Micro-farming system prevailing inBarua village was identifiedas rain-fed pond aquaculture system. All the fishponds in the village are rain fed. The small ponds are seasonal where water is retained for 6-8 months, i.e. from June/July to January/February. The large and medium ponds are perennial where water is retained throughout the year. Though traditional fish farming is very much prevalent in the area, the concept of scientific farming is catching up slowly. The farmers normally rear Indian carps like catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), bata (*Labeo bata*) in their ponds. They occasionally include exotic carp species like silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys nobilis*) in the ponds. Other local fish species like climbing perch (*Anabas testudineus*), magur (*Clarius batrachus*), singhi (*Heteropneustes fossilis*), murrels (*Channa sps.*), *etc.* are also reared in the ponds. Apart from culturing fish, harvesting of miscellaneous fish from paddy fields is another practice in the area. During monsoon, when the ponds get filled with rain water, the brood fish (mainly air-breathing species) escape in to nearby paddy fields where they breed The young seedlings growin the paddy fields for 3-4 months, till the paddy is harvested.

Problem-Cause Diagram for low fish yield: During the analysis of micro-farming situations, emphasis was given to identify the problems faced by the farmers of Barua village in traditional pond fishery. The identified problems were subjected to problem-cause analysis through causal diagram in order to decide on the technological interventions for their solution. Analysis of problem-cause diagram revealed that unscientific stocking density, improper species combination, lack of integration with livestock, non-inclusion of high-value species like prawn, non-use of supplementary feed etc. are the important reasons for low yield of fish and low profitability from fish farming system of the locality. The socio-economic and bio-physical constraints relating to low fish yield of fish and low profitability of farmers have been depicted in the following problem cause diagram

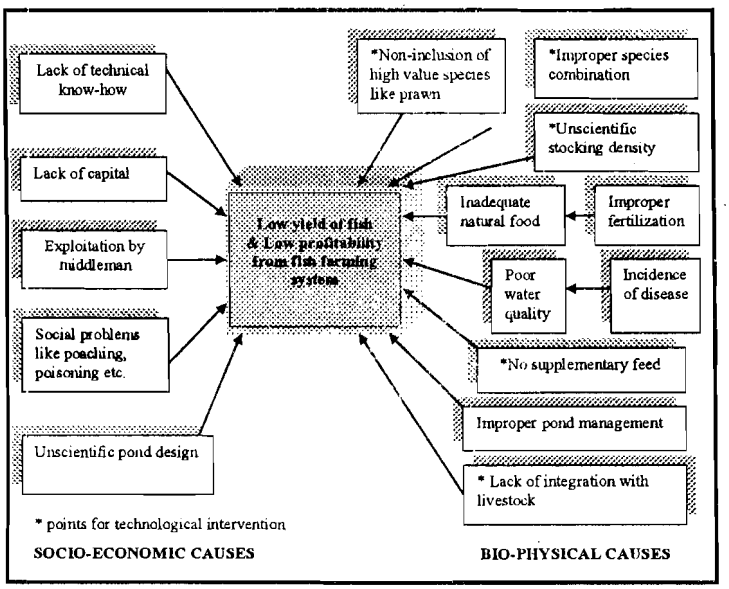

Fig. 2: Problem cause diagram for lowfish yield and low profitability

Technological interventions: Four numbers of technological interventions were designed with farmer's participation to solve the problems related to low yield of fsh and low profitability from fish farming system in the locality. Above interventions were implemented in farmers' ponds either in form of verification trial (VT) or On-farm Trial (OFT). Under each intervention, 10 ponds were covered and hence the figures presented in the table 6 & 7 are the average of 10 data. Irrespective of intervention, no notable change in any water parameter was observed except temperature and water depth. However, growth and total production of fish varied in different interventions. Optimum stocking of fingerling@ 8000/ha. (intervention no. 1) increased the fish production by 57% in the village with average yield of 2506 kg/ha/ crop. The farmers earned gross income of Rs.1,25,300/ha./crop and net profit of Rs.62,650/ ha./crop with the benefit-cost (B.C) ratio of 2.00. By integrating duck @ 250 / ha. with fishery (intervention no. 2), the farm profitability increased by 125% with fish production of 3596 kg/ha./crop. Under this intervention, the farmers achieved gross income of Rs.1,89,800/ha./crop and net profit of Rs. 1,22,500/ha./crop with B.C. ratio of 2.82. By using supplementary feed of rice bran and oil cake in 1:1 ratio (intervention no. 3), higher fish production of 3332 kg/ha./crop was obtained with feed conversion ratio (FCR) of 2.95. In this intervention gross income of Rs.1,66,600/ha./crop and net profit Rs.88,016/ ha./crop with B.C. ratio of 2.12 were recorded. Inclusion of *M. rosenbergii* in carp farming system (intervention no. 4) resulted in 92% increase in farm income with fish yieldof 2184 kg/ha./crop and prawn yield of 122 kg/ha./crop.

Table 6: Water quality parameters* recorded in fishponds under different interventions

Parameters	Control	Intervention 1	Intervention 2	Intervention 3	Intervention 4
Temp. (^{0}C)	28.8 (18.6-38.6)	28.4 (18.3-38.2)	28.9 (18.1-39.2)	28.0 (18.2-37.8)	28.8 (18.4-38.6)
pH	8.2 (7.7-8.7)	8.7 (7.9-9.2)	8.5 (7.8-9.1)	8.3 (7.7-8.8)	8.6 (7.9-9.0)
Dissolved Oxygen (mg/l)	5.1 (4.2-5.7)	5.4 (4.8-6.1)	5.9 (5.1-6.4)	5.5 (4.8-6.2)	5.5 (5.0-6.0)
Transparency (cm)	38.2 (29-42)	33.7 (23-38)	34.2 (22-40)	33.6 (20-41)	35.9 (24-41)
Water Depth (cm)	115 (98-132)	109 (94-128)	114 (100-129)	123 (104-136)	119 (101-140)

* Mean of the monthly observation recorded throughout the culture period
Figures in the parenthesis represent the range of values

Table 7: Impact of technological interventions on different performance indicators

Parameters	Control	Intervention 1	Intervention 2	Intervention 3	Intervention 4
Average growth of fish in nine months (g)	450	600	710	703	620-Fish 50-Prawn
Daily wt gain (g/day)	1.48	2.22	2.62	2.60	2.30-Fish 0.71-Prawn
Average fish yield (kg/ha./crop)	1600	2506	3596	3332	2184 Fish + 122 kg prawn
Gross income (Rs./ha./crop)	64,000	1,25,300	1,89,800	1,66,600	1,22,680
Cost of prod^n (Rs./kg)	27.50	25.00	18.71	23.58	24.85
Expenditure (Rs./ha./ crop)	44,000	62,650	67,300	78,584	54,283
Net Profit (Rs./ha./ crop)	20,000	62,650	1,22,500	88,016	68,400
B.C. ratio (Income:expense)	1.45	2.00	2.82	2.12	2.26

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CHEMICAL MANIPULATION OF SEED INVIGOURATION AND PLANT POTENTIATION OF TWO PROMISING PULSE CROPS UNDER STRESSFUL STORAGE CONDITION

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Abstract ■ An investigation was carried out on maintenance of seed invigouration and plant potentiation of pea and green gram cultivars by using some selected chemicals. Pretreatment of pea (*Pisum sativum* L. cv. BIOL-185) and green gram (*Phaseolus mungo* L. cv. WB-13) seeds with aqueous solutions of sodium dikegulac (Na-DK, 2, 3:4, 6-di-O-isopropylidene-a-L-xylo-2-hexalo furanosate) and ascorbic acid (100 μg ml^{-1} of each) for 6 hours before accelerated ageing treatment (99.5% RH and 32±2°C) for different durations (0 to 30 days) slowed down the rapid loss of germination and reduced the time (h) required for 50% seed germination (T_{50}) in both the species. The chemicals also significantly arrested profuse leakage of amino acids from seeds. Concomitantly, the reduction of protein, insoluble carbohydrate, DNA and RNA levels as well as activity of catalase enzyme of seed kernels during forced ageing period was ameliorated to a significant extent in the chemical-pretreated seed lots of the two species. Conversely, ageing-induced stimulation of the activity of amylase enzyme was alleviated by the seed pretreating agents. Again, potted plants raised from the pretreated seeds showed better performance, measured in terms of some vital physiological and biochemical parameters. The promising effects of the experimental chemicals on storage potentiation of the seeds and enhancement of plant vigour are apparent in this investigation. Thus, the promising effects of the selected chemicals on storage potentiation of the seeds and enhancement of plant vigour are apparent in this investigation.

Key words: Pea, green gram, Na-DK, ascorbic acid, storage potentiation, plant vigour, accelerated ageing.

Introduction

Storing of seeds is a serious problem in tropical and subtropical countries where high temperature and high relative humidity greatly accelerate seed ageing phenomenon causing consequent deterioration and non-viability of seeds. The problem of retention of seed vigor in many states of India is much more acute

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because of its semiarid climate where high relative humidity prevailing during the major part of a year is very conducive to the growth of microorganisms, particularly fungi.(Christensen and Kaufmann 1965, Aziz and Shahir, 1997).These two environmental factors strongly impair seed and seedling health and cause to reduce percent seed germinability and seedling performance at a rapid rate (Copeland and McDonald,1995,Pati,and Bhattacharjee,2008). Thus, Indian cultivators are very often compelled to use low vigour seeds in agriculture. To get rid of this problem, strategies are now being undertaken to improve the storage potential of seeds for enhancing their life span (Chhetri *et al.*, 1993, Basu, 1994, Pati *et al.*, 2004).

Keeping in mind the problem of seed storing in our country, an attempt has been made in this investigation to prolong the storage life of the pea and green gram seeds having viability problems using sodium dikegulac (Na-DK, 2, 3:4, 6-di-O-isopropylidene-a-L-xylo-2-hexalo furanosate) and ascorbic acid. Experiments of this investigation were carried out under accelerated ageing condition to obtain more or less uniform and expeditious results. In fact, accelerated ageing treatment, as imposed by high temperature and high relative humidity (RH), provide a powerful tool for studying the process of seed deterioration over a very short period (Heydecker, 1972, Pati, 2007) and this mimics the natural ageing process.

Thus, the prime objective of this work is to probe the efficacy of the test chemicals on seed invigouration of a pea and a green gram cultivar by analysing germination behaviour, TTC stainability, metabolic status of seeds and plants.

Materials and Methods

After surface sterilization (0.1% $HgCl_2$ for 90 seconds) the seed samples of pea (*Pisum sativum* L. cv. BIOL-185) and green gram (*Phaseolus mungo* L. cv. WB-13)were separately presoaked in aqueous solutions of sodium dikegulac (Na-DK, 2, 3:4, 6-di-O-isopropylidene-a-L-xylo-2-hexalo furanosate) and ascorbic acid (100 µg ml^{-1} of each), or distilled water for 6 hours (h) and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemicals present in the aqueous solution. The pretreated seed lots (200 g for each treatment) were taken in separate cloth bags and thus stored in a desiccator in which 99.5% relative humidity (RH) was preimposed by keeping 250 ml 1.57% H_2SO_4 within it. This experimental set up was kept at 32±2°C for 30 days allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 15 day intervals to restore the desired RH within the desiccator for 30 days. To analyse the percentage germination, four groups of 100 seeds i.e. 400 seeds of each treatment were transferred to separate Petri dishes containing filter paper moistened with 10ml distilled water. Germination data were recorded after 96 h of seed soaking following the International Rules for SeedTesting (ISTA, 1976). The time for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al.* (1984). For recording TTC (2, 3, 5-triphenyl tetrazolium chloride) stainability, dehusked seeds of each treatment were allowed to imbibe 0.5% (w/v) TTC solution in Petri dishes and kept overnight in dark. Percentage TTC stained seeds (deep red) was calculated from the total number of seeds of each treatment.

Leaching of amino acids from seeds was analysed after immersing 1g of seeds in 10ml deionised distilled water for 24 h. Protein, insoluble carbohydrate, DNA and RNA contents as well as activities of catalase and amylase enzymes were analysed from seed kernels of each sample. Quantification of

insoluble carbohydrates and amino acid was done following the method of McCready *et al.* (1950) and Moore and Stein (1948) respectively. Protein and nucleic acids (DNA and RNA) levels were estimated as per the methods of Lowry *et al.* (1951) and Cherry (1962) modified by Choudhuri and Chatterjee (1970) respectively. Extraction and estimation of the enzyme catalase was made following the method of Snell and Snell (1971) as modified by Biswas and Choudhuri (1978). Amylase activity was estimated as per the methods of Khan and Faust (1967). For assaying these enzymes, the blank was taken as zero time control and the activity was expressed as $(\Delta ODxT_V)/ (txv)$, where ΔOD is the difference of OD of the blank and sample. T_V is the total volume of filtrate, t is the time (min) of incubation with the substrate and v is the volume of filtrate taken for incubation (Fick and Qualset, 1975).

Levels of chlorophyll, protein and activity of enzyme catalase were analysed from the leaves of 30 days old plants, raised after 0 and 30 days of seed ageing. To study the health status of plant, root length, shoot length, stem diameter and internodal elongation were recorded from 10 uniformly grown plants of each treatment. Data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits (Panse and Sukhatme, 1967).

Results and Discussion

Data clearly revealed that pretreatment of the seed species with aqueous solutions of Na-DK and ascorbic acid significantly alleviated the accelerated ageing-induced loss of germination and reduced T_{50} hours (Table 1), increased TTC stainability (Table 2), slowed down the rapid leaching of amino acids and insoluble carbohydrates (Table 3), alleviated the loss of protein (Table 2), nucleic acids (Table 4) as well as catalase and amylase (Table 5) enzymes.

Seeds were presoaked with the aqueous solution of the chemicals or distilled water for 6h and then dried back to original seed weight. This was repeated twice. Pretreated seed samples were kept under 99.5% RH and data were recorded after zero (0), 15 and 30 days of accelerated ageing.

In leaves, chlorophyll and protein levels as well

Table 1. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on percentage seed germination and T_{50} (time required for 50% germination) values of pea and green gram seeds.

Seed samples	Treatments	Percentage seed germination			T_{50} of germination		
		Days after accelerated ageing					
		0	15	30	0	15	30
Pea	Control	100	78	38	12	36	NA
	Na-DK.	100	84	57	12	24	72
	Ascorbic acid	100	80	52	12	24	84
	LSD (P = 0.05)	NC	5.60	4.58	NC	2.50	6.05
Green gram	Control	100	62	31	12	30	NA
	Na-DK.	100	78	56	12	24	78
	Ascorbic acid	100	71	50	12	24	90
	LSD (P = 0.05)	NC	5.25	3.50	NC	2.01	5.72

NC: Not calculated; NA: Non attainment of 50% germination.

Table 2. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on TTC stainability and protein (mg/g fr. wt.) levels of pea and green gram seeds. Treatments and recording of data as in Table 1.

Seed samples	Treatments	Percentage TTC-stained seeds			Protein		
		Days after accelerated ageing					
		0	15	30	0	15	30
Pea	Control	100	80	42	71.10	50.21	21.96
	Na-DK	100	86	61	71.70	61.80	41.03
	Ascorbic acid	100	83	59	71.17	57.82	33.11
	LSD (P = 0.05)	NC	NS	4.05	NS	4.20	2.17
Green gram	Control	100	70	39	61.31	40.33	19.87
	Na-DK	100	79	58	61.42	51.99	31.26
	Ascorbic acid	100	73	54	61.09	48.12	28.99
	LSD (P = 0.05)	NC	5.80	3.14	NS	3.77	1.58

NC: Not calculated; NS: Not significant.

as activity of catalase (Table 6) were remarkably declined in leaves of plants raised from seeds pretreated with the aqueous solutions of Na-DK and ascorbic acid as well as in control samples. However, the declining magnitude was found to be much less in the pretreated seed samples. Again, the accelerated ageing treatment for 30 days resulted in significant reduction of root length, shoot length, stem diameter and internodal elongation of plants raised from both the plant extract pretreated and control seed samples (Table 7). Surprisingly the Na-DK and ascorbic acid stimulated the overall growth parameters of plants.

The proposal that a decrease in membrane lesions might play a significant role in deterioration of seeds has been supported by the work on solute leakage accompanying a loss in germinability and viability (Powell and Matthews, 1977, Pati, 2007). The ability of seeds to recognize its membrane rapidly as the desiccated tissue rehydrates is a crucial factor for successful germination and this is clearly documented in the literature (Simon, 1974). Much evidence has been put forward to suggest that membrane status within the germinating embryo is an important factor in deterioration (Ponnachan *et al*, 1993, Kamalakkannan and Stanely, 2003). Thus, in

Table 3. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on leachate amino acids (mg/g/10ml) and insoluble carbohydrates (mg/g fr. wt.) levels of pea and green gram seeds.
Treatments and recording of data as in Table 1.

Seed samples	Treatments	Leachate amino acids			Insoluble carbohydrates		
		Days after accelerated ageing					
		0	15	30	0	15	30
Pea	Control	32.0	52.6	67.9	23.10	18.59	10.19
	Na-DK	31.2	38.2	42.6	23.17	21.82	18.02
	Ascorbic acid	31.7	39.9	48.9	23.16	20.19	17.07
	LSD (P = 0.05)	NS	3.05	4.15	NS	1.01	0.08
Green gram	Control	37.2	57.9	72.1	21.81	16.42	11.82
	Na-DK	38.5	40.7	51.9	21.87	19.66	18.01
	Ascorbic acid	38.2	49.3	54.1	21.86	18.53	16.74
	LSD (P = 0.05)	NS	3.50	4.99	NS	1.05	0.69

NS: Not significant.

Table 4. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on DNA (µg/g fr. wt.) and RNA (µg/g fr.wt.) levels of pea and green gram seeds. Treatments and recording of data as in Table 1.

Seed samples	Treatments	DNA			RNA		
		Days after accelerated ageing					
		0	15	30	0	15	30
Pea	Control	48.0	39.2	30.9	126.1	78.2	39.0
	Na-DK.	48.8	50.1	40.0	126.3	102.1	51.9
	Ascorbic acid	48.6	42.4	36.2	126.6	96.7	48.2
	LSD (P = 0.05)	NS	3.27	2.96	NS	7.55	2.99
Green gram	Control	42.9	55.5	28.2	104.2	72.9	28.6
	Na-DK.	42.7	49.2	35.2	104.0	98.2	49.9
	Ascorbic acid	42.0	41.7	30.9	104.6	92.4	48.0
	LSD (P = 0.05)	NS	4.05	2.25	NS	6.50	2.20

NS : Not significant.

the present study ageing-induced higher leaching of amino-acids with concomitant reduction of seed germinability and TTC stainability are indicative of damage of seed membrane and consequent loss of seed vigour and viability. The chemical-induced substantial amelioration of all these deleterious effects are indicative of seed potentiation under adverse storage environment.

The results therefore point out that although deterioration is a common phenomenon in treated and control samples of the two seed species, the catabolic processes within the treated seed samples remained somewhat subdued, thereby rendering them tolerant against unfavourable storage environment.

Table 5. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on activities of enzyme catalase (ΔODxTv/txv) and amylase (ΔODxTv/txv) of pea and green gram seeds.
Treatments and recording of data as in Table 1.

Seed samples	Treatments	Catalase			Amylase		
		Days after accelerated ageing					
		0	15	30	0	15	30
Pea	Control	42.0	29.0	18.8	38.9	48.9	69.2
	Na-DK.	42.1	38.1	32.2	38.0	41.2	50.0
	Ascorbic acid	42.0	36.6	28.9	38.7	43.0	52.1
	LSD (P = 0.05)	NS	2.17	1.50	NS	3.28	4.39
Green gram	Control	40.4	26.2	16.9	37.1	50.0	67.8
	Na-DK.	40.2	32.1	27.9	37.2	40.1	51.7
	Ascorbic acid	40.0	30.9	25.0	37.0	41.2	53.4
	LSD (P = 0.05)	NS	2.50	1.35	NS	3.95	4.80

NS : Not significant.

Table 6. Effect of seed pretreatment with aqueous solutions of Na-DK and ascorbic acid (100 µg ml^{-1} of each) on chlorophyll (CHL; mg/g fr. wt.), protein (PR; mg/g fr.wt.) and catalase activity (CAT; ΔOD x Tv/txv) from leaves of plants (30 days old) raised from seeds which underwent accelerated ageing for 0 and 30 days of pea and mung bean cultivars.

Seed samples	Treatments	Days after accelerated ageing					
		0			30		
		CHL	PR	CAT	CHL	PR	CAT
Pea	Control	23.0	67.0	36.9	12.2	38.9	11.2
	Na-DK.	26.1	70.9	38.8	18.1	49.2	22.1
	Ascorbic acid	26.0	70.0	37.2	17.5	47.0	20.2
	LSD (P = 0.05)	0.23	5.90	3.05	1.01	3.40	1.02
Green gram	Control	22.0	85.0	37.0	10.2	30.6	10.2
	Na-DK.	24.1	90.1	38.2	13.9	46.0	18.3
	Ascorbic acid	23.9	89.9	38.0	13.7	45.9	17.9
	LSD (P = 0.05)	1.98	7.80	3.25	0.09	2.80	1.01

Available reports show that during seed ageing a loss of some vital cellular components including protein, carbohydrates, nucleic acids occurred (Abdul Baki and Anderson, 1972, Kole and Gupta, 1982). Catalase is regarded as a scavenger enzyme (Fridovich, 1976) and higher activity of this enzyme is indicative of higher plant vigour (Pati and Bhattacharjee, 2008). In this investigation, the chemical-induced arrestation of rapid loss of the enzyme activity is indicative of strengthening the defence mechanism by the chemicals under adverse storage condition.

It can be concluded from the results of this investigation that aqueous solutions of Na-DK and ascorbic acid are effective in enhancing storage potential of pea and green gram seeds. Thus, invigouration property of the present seed pretreating agents seems to be apparent from our experimental results.

Table 7. Effect of seed pretreatment with leaf extracts of *Aegle* sp. and *Vitex* sp. (50g/ 500ml each) on root length (RL; cm), short length (SL; cm), stem diameter (SD; cm), internodal elongation (IE; cm) of plants (30 days old) which raised from seeds which underwentaccelerated ageing for 0 and 30 days of pea and mung bean cultivars.

Seed samples	Treatments	Days after accelerated ageing							
		0				30			
		RL	SL	SD	IE	RL	SL	SD	IE
Pea	Control	5.3	21.6	1.6	1.2	3.1	9.6	0.7	0.6
	Na-DK.	5.4	22.0	1.6	1.2	4.2	11.6	1.0	1.0
	Ascorbic acid	5.5	21.7	1.6	1.2	4.0	11.5	1.0	1.0
	LSD (P = 0.05)	NS	2.01	NC	NC	0.03	0.91	0.06	0.06
Green gram	Control	5.6	18.7	0.9	1.6	2.8	7.2	0.5	0.8
	Na-DK.	5.7	19.1	1.2	1.7	4.0	9.0	0.9	1.1
	Ascorbic acid	5.6	19.2	1.2	1.7	3.8	8.1	0.9	1.0
	LSD (P = 0.05)	NS	NS	0.08	NS	0.21	0.66	0.05	0.06

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EVALUATION OF CARDIOVASCULAR STRESS OF UNDER-GROUND AND SURFACE COALMINE LOADERS

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Abstract ■ Coalmine loaders work in underground mines as well as on the surface mines. Both male and female loaders are found to work on the surface but only male loaders work in underground coalmine. The aim of the present study was to evaluate the cardiovascular stress of the loaders. The study was conducted with 27 coalmine loaders. The resting and working heart rate of the subjects were recorded by a polar heart rate monitor. The cardiovascular stress index (CSI) was determined from resting, working and age predicted maximum heart rate. The results revealed that the mean working heart rate and maximum heart rate was significantly higher ($p<0.05$) in underground loaders than that of surface loaders. Among the surface loaders there was no significant difference in working and maximum heart rate between male and female subjects, except in resting heart rate. The CSI was higher in underground coalmine loaders than that of surface loaders. The CSI was compared with other groups of loaders. It was concluded that the higher work load and environmental stress might be the related to the higher cardiovascular stress in the underground coalmine loaders.

Introduction

Coal industry is one of the most vital industries in India. India has a long history of commercial coal mining. Basket loading is a common manual task in coal mines. It exposes the loaders to unique physical and environmental demands, leading to high risk involvement of suffering from occupational health disorders. According to NIOSH (2000), cumulative trauma disorders continue to constitute the largest category of occupational disease in mining and often result in prolonged disability. The work related health hazards may be related to the various kinds of job related stresses including environmental stress, postural stress and physiological stress. The evaluation of the physiological cost of coal mine loaders has been investigated a little in India. Sporadic studies revealed that the workload of basket loaders vary from heavy to very heavy (Bhatnagar and Drury, 1985, CMRS, 1993; and Paul et al. 2003). Pal (2003) reported that individual task analysis for oxygen uptake cum calorific expenditure and analysis of typical work exposure can provide an estimate of stresses imposed on the body during the performance of an underground task.

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NIOSH (National Institute of Occupational Health and Safety, 2009) defines work stress as the harmful physical and emotional responses that occur when the requirements of the job do not match the capabilities, resources or needs of the worker. There are one million strokes, six million hospitalizations and the total costs of cardiovascular disease (CD) in 2005 were estimated at 400 billion USD. Etiologic research has demonstrated a strong relationship between workplace stressors and adverse health outcomes, notably cardiovascular disease (CVD) (Belkiæ et al, 2004 and Kivimäki et al, 2006).

Physiological effects of work stress have also been studied by some researchers. Changes in heart rate were sporadically studied in mining occupation (Bhatnagar & Drury, 1985; CMRI, 1993; Paul et al, 2003) and consistently in other occupations (Kang et al, 2005; Vrijkotte et al, 2000; Cheng et al. 2006; Comtois, 2008).

Occupational stress is being associated with cardiovascular risk factors since long. Vast literature is available confirming an association of high job strain and cardiovascular diseases (Bosma et al, 1998; Kivimaki et al, 2002; Väänänen , 2008; Vrijkotte et al, 2000).

The coal mine loaders are engaged in work in underground and on surface. Only male miners work in the underground mines whereas both men and women workers are found to work on the surface mine. In both underground and slurry loading tasks, one loader cuts the slurry/ coal and fills the cane basket and the other loader waits in front of the basket waiting for his basket to be filled. After filling, the loader who was engaged in filling the basket helps the other loader in lifting the load from ground level to overhead of the other loader. The second loader carries the load to the truck (during slurry loading) or coal tub (during coal loading) and empties the basket and returns to the loading point. Here again his basket is refilled and it is again lifted with the help of the other loader to place the basket overhead.

A large number of loaders are engaged in loading of coal or slurry. The present study was aimed to evaluate cardiovascular stress of underground and surface loaders of coalmines.

Methodology

The study was approved by the Human Ethical Committee of the present institute. The study was carried out in the coal mines, which were located in Dhanbad district of Jharkhand state. Twenty seven basket loaders of those coal mines volunteered in the study. The workers, who were suffering from chronic or acute diseases, were excluded from the study. The basket loaders in underground were engaged in loading of coal and the loaders in the surface were engaged in loading slurry.

Anthropometric data:

The height and weight of the subjects were measured with minimum clothing and using standard landmarks. The body mass index (BMI) was computed by using the following formula: BMI= weight (kg) / height^2 (meter).

Evaluation of Cardiovascular Stress

Heart rate (HR) was considered as a parameter to assess the cardiovascular response of coal mine loaders. Heart rates of surface and underground mine loaders were recorded in beats per min (bpm) using POLAR Heart Rate Monitor at rest (for 15 minutes) and at work (for 50 to 85 minutes). The working heart rate during 1st loading cycle, mid-loading cycle and the final loading cycle were recorded. Mean heart rates during rest and work were computed.

Cardiovascular Stress Index (CSI) was computed using the following formula (Trites et al, 1993) to evaluate the degree of cardiovascular stress of the basket loaders. The resting heart rate (HR_{rest}), mean working heart rate (HR_{work}) and maximum heart rate (HR_{max})

were used for computing the index.

$$CSI = 100 \times [(HR_{work} - HR_{rest}) / (HR_{max} - HR_{rest})$$

Where, HR_{max} = 220 - age (in years)

Measurement of load: The amount of load handled and distance covered by the loaders was recorded. A weighing machine and large plastic tape were used for this purpose.

Results :

The height, weight and BMI of the male and female coal mine loaders have been presented in Table1. The results showed that the height and weight of the male coal mine loaders were significantly higher than that of the female loaders. The BMI values showed that the both male and female loaders were under the 'normal weight' categoryaccording to the classification of WHO (2000). From the analysis of data it was further noted that most of the male loaders (88.4%) and female loaders (70%) were within the normal range. The percentage of underweight and overweight subjects was low among the male and female loaders. There was no significant difference in anthropometric parameters between underground and surface male workers.

The cardiovascular parameters of the underground and surface loaders of coalmines have been presented in Table 2. The mean working heart rate of male coal mine loaders was found to be 38.8% and 40.12% of the maximum heart rate of the surface loaders and the underground loaders respectively. In case of female loaders, the mean working heart rate was 37.2% of the maximum heart rate.

It was revealed (Table 2) that there was a significant difference in resting heart rates between the male and female slurry loaders ($P<0.05$) working on the surface. The working heart rate of male surface loaders was found to be lesser, although non-significantly, than that of the female loaders. The mean working heart rate was significantly higher ($p<0.05$) in underground loaders than that of surface male loaders. The maximum heart rate was significantly different ($p<0.01$) between male surface and underground loader.

Table 1: Height, weight and BMI of male and female basket loaders of coal mines [Mean (SD)]

Gender	Sample size	Height (cm)	Weight (kg)	BMI (Kg/m^2)
Male	17	161.82 (6.3)	58.19 (8.00)	20.65 (3.38)
Female	10	150.40 (5.9)**	48.22 (7.97)**	21.90 (3.06)

**$p<0.01$

Table 2: Different heart rates (bpm) of loaders.

Parameters	Surface Loaders		Underground Loaders
	Male (n=10)	Female (n=10)	Male (n=7)
Resting heart rate	78.3 (11.0)	85.0 (4.7)*	74.6(6.5)
Mean working heart rate	113.9 (4.9)	116.9 (7.9)	117.8 (5.3)*
Maximum heart rate	186.1 (12.7)	186.1 (3.98)	196.7(1.9)**

Note: Values are Mean (SD)

*$p<0.05$; ** $p<0.01$ with reference to male surface loader

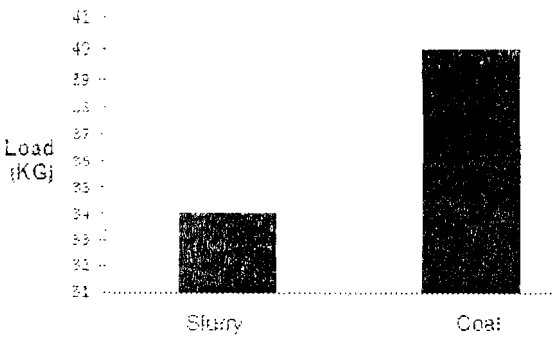

Fig 1: Mean amount of load handled by the loader during slurry (surface) and coal loading (underground)

different studies. There was a significant difference in the resting heart rate and peak working heart rate between the present study and other study (Dey et al, 2007). Such difference might be due to difference in the age and activity level of the loaders.

The cardiovascular stress index (CSI) of the coalmine loaders has been presented in Table 4. the highest extent of CSI was observed in male underground loaders (34.32) and it was slightly lower in male surface loaders (33.06)

The CSI of the coalmine loaders was compared with that of other groups of workers, viz., agricultural workers, steel workers, cane cutters and car assembly workers. The results showed that the CSI of underground coal mine loaders were higher (Table 5) than that of other groups of workers, except silviculture workers (Trites et al. 1993).

Table 3: Comparison among load handlers mentioned in various studies conducted in the eastern coalfields.

Study	Sample size	Age (yr)	Resting heart rate (bpm)	Peak heart rate (bpm)	Workload
Paul et al. 2003	4	-	86.0	132.0	Heavy to very heavy
Dey et al. 2007	12	35.6 (8.3)	64.0 (5)*	184.0 (8.2)*	Heavy to extremely heavy
Present study	17	23.3 (1.9)	74.6 (6.5)	138.0 (9.2)	Heavy to very heavy

Note : Values are Mean (SD)
*p<0.001 with reference to present study

The underground loader had to carry greater amount of load than that of surface loaders (fig.1). The mean values of resting heart rate, peak working heart rate and workload assessment of underground loaders were compared among different studies in eastern coalfields (Table 3). The resting and peak heart rates had variations among the loaders of

Table 5 : CSI of loaders engaged in different occupations

Coalmine (Present Study)	Agriculture (Ghosh et al. 2003)	Silviculture (Trites et al. 1993)	Steel (Vitalis et al. 1994)	Cane cutters (Vitalis et al. 1994)	Car assembly (Goldsmith, 1978)
34.32 (6.47)	23.18 (9.49)	39.2 (4.0)	25.0 (14.0)	33.0 (4.7)	20.0 (7.0)

Discussion

Cardiovascular fitness determines the endurance capacity of an individual. Heart rate is said to reflect the amount of work the heart must do to meet the increased demands of the body when engaged in activity, for which comparison of the resting heart rate is essential with working heart rate. In the present study, the physiological work load of the coalmine loaders was determined on the basis of heart rate responses.

The mean working heart rate of loaders was found to vary from 113.9 to 117.8 bpm. According to the classification of Astrand and Rodalh (1970), on the basis of working heart rate, workloads of both male and female loaders were 'heavy'. Similar results of workload analysis was obtained by Paul et al (2003) while analyzing posture and workload of loaders in a few underground coal mines of central part of India. They reported that the workload of loaders during loading of coal in the basket varied from heavy to very heavy based on given set of working conditions

Tersman et al. (1991) reported that heart rate in females was more than males during stress condition. The mean working heart rate was significantly higher ($p<0.05$) in underground loaders than that of surface male loaders. The higher working heart rate in underground loader might be due higher work load imposed on the underground loaders than that of surface loaders. In the present study, the average weight handled by the surface loaders was 34.4 kg of slurry where as average weight handled by underground male loaders was 40.6 kg of coal (Fig. 1). The average distance traveled by the loader in underground was about 25.57 meters during filling the tub with coal. This distance was more than the distance covered by the slurry loaders (average of 8.0 meters). So the workload of the underground loaders was more compared to those slurry loaders. Other investigators (Kadoya et al, 2010; Bates and Schneider, 2008) opined that increased physical and physiological stress on the loaders was related with level of fatigue, aerobic strain and physical workload.

The higher maximum heart rate of underground loader might be due to higher mean age (33.9 ± 3.9 yrs) than that of the surface loaders(23.3±1.9 yrs). Similar observations were also made by CMRS (1993) and Paul et al (2003). Pal (2003) reported that environmental stress was high in underground mines, which contributed to the physiological stress and there by an increase in cardiac cost. As the environmental stress in the underground condition is generally greater (CMRS, 1993), relatively younger loaders might be able to withstand more stressful condition in the underground.

The difference of CSI noted among the underground and surface loaders can be attributed to the nature of work performed by these loaders under extreme environmental conditions. In the underground mines, the work was conducted in a confined space and under thermal stress (CMRS, 1993). The hot and humid temperature might impose cardiac stress on the loaders. Similar results were obtained by Dey et al (2007). But the increased cardiovascular stress index noted among the male slurry loaders in the present study can be attributed to the nature of work they perform efficiently.

The difference in CSI among different groups of workers might be due to difference in nature and heaviness of work, duration of work and environmental conditions. It was established that the heart rate in terms of relative cardiac cost is a reliable measure to rationalize the physiological workload since the later showed better correlation with the relative aerobic strain (Dey et al, 2006; Ahonen et al, 1990).

Conclusion

It may be concluded that the cardiovascular stress of the underground coalmine loaders was higher than that of surface loaders. The increased cardiovascular stress in the underground might be related to the amount of load handled in the work shift, and nature of load handling in underground mines. The coal mine workers had higher magnitude of cardiovascular stress than that of other category of agricultural or industrial workers.

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EFFECTS OF TANNERY EFFLUENTS ON GERMINATION AND SEEDLING GROWTH OF SOME CROPS

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Abstract ■ The effects of effluents from leather tanning industry on seed germination of boro rice (BARI dhan-29), chickpea (BARI chola-4) and wheat (satabdi) were studied. Results revealed that tannery effluents had inhibitory effects on seed germination and seedling growth on test crops. Although chickpeas were found to some extent tolerant but wheat and rice were very much sensitive with respect to germination. Such germination was observed relatively better when isolated bacteria with small quantity of glucose were released in the effluents. It proves that released bacteria can detoxify the toxic pollutants of the effluents and suppress their deleterious effects.

Key words : Effluents, tannery, bacteria, seeds, germination

Introduction

Dhaka, a megacity of Bangladesh, is one of the most densely populated cities in the world. In the south-west part of the city, there is a tannery area consisting of about 206 tanning industries occupying 25 hectares of land at Hazaribagh. Leather, a traditional export item in Bangladesh, enjoys a good reputation world wide for its quality. This sectors plays a significant role in the economy of Bangladesh interms of its contribution to export and domestic market. The tanning industries of Hazaribagh are processing some 220 metric tons of hide a day with an associated release of 600-1000 kg of solid waste per ton resulting from processed hide (Zahid *et al.*, 2004). Daily discharge of wastes from these tanneries is about 18000 liters of liquid wastes, 115 tones of solid wastes during peak time and 75 tones during off-peak time. About 60,000 tones of raw hides and skins are processed every year, a process which releases nearly 9,500 liters of untreated effluent in to the open environment daily (Rusal *et al.*, 2006). Industrial effluents contain various poisonous salts, alkalizes, acids gases, heavy metals (chromium, cadmium, lead

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etc.) and colored pigments. These polluted effluents are thorn into the canals, streams or rivers where they deteriorate the quality of water making the water unfit for irrigation purposes and for the use of animals. The harmful effects of effluents and waste products from different industries have been reported by Tripathi (1978), Chain *et al.* (1987) and Chadderton (1988).

Heavy metals are one of the main sources of environmental pollution, which becomes the greatest risk to the plant life (Fernandes and Henriques, 1991). Heavy metals like chromium, cadmium and lead, which are used in tanning industry and disposal from the industry effluents. These metals reduce the growth of plant, decreased net photosynthesis and the biomass of plants. Heavy metals persist indefinitely in soil thereby posing an ever-increasing threat to human health and agriculture (Leyval *et al.*, 1995). Cleanup processes of heavy metal pollution are expensive and environmentally destructive (Nanda *et al.*, 1995). Recently, Scientists have started to generate cost-effective technologies that include the use of microorganisms, biomass and live plants in cleaning process of polluted areas (Boyajian and Carreira, 1997; Wasay *et al.*, 1998).

Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species (Claire *et al.*, 1991). The use of wastewaters effluents for agriculture is created to various problems like soil salinity, interaction of chemical constituents of the wastes with the uptake of nutrients and changes in soil property and micro flora (Goel and Kulkarni, 1994). This necessitates a detailed scientific study before any specific waste can be used for irrigation for a particular crop with particular soil and climate.

In Hazaribagh and surrounding areas, many vegetables farms are irrigated with the waste water from polluted Buriganga rivers. Studies by FAO/WHO (1993) have found that metal concentrations are high and with increased consumption of these vegetables, future health problems for consumers are inevitable. Severe effects include reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as mercury and lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells. This can lead to joint diseases such as rheumatoid arthritis, and diseases of kidneys, circulatory system, and nervous system. Since, information on effect of tannery effluents on seed germination is inadequate or scarce in Bangladesh, therefore, this study was undertaken to investigate the toxicity of effluents and their effects on seed germination and seedlings growth of boro rice, wheat, chickpea and also to know the role of microorganisms in the bioremediation of toxic pollutants through seed germination.

Methods

The experiment was conducted in the Department of Zoology, University of Rajshahi, Rajshahi during the period from March to June. Sample of effluents were collected from tanning industry (Hazaribagh, Dhaka) and used as sources of inocula for isolation of microorganisms. The sample was taken in 10-liter capacity polyethylene containers directly from the outlet of the factories at randomly selected points. The effluents were light green color and had a bad smell. The collected effluents were stored in dark at room conditions for future uses.

Soil samples were collected in polyethylene bags with the help of spade from four corner of effluents dumping areas of Hazaribagh.

Surface soil were collected and dried at room temperature (30° 3°C) and finely ground (<0.1 mm). One gram of soil was then burnt into ashes in a crucible. These ashes were taken and moistened with a little double distilled water. Concentrated HNO_3 and HCl were added successivdy in a ratio of 3:1. Soil sample in the beaker were then heated gently on a heating plate until the samples were digested, which was indicated by the formation of a clear solution above the soil residue. After dilution and filtration the digested solution was analysed for determination of heavy metals by Atomic Absorption Spectrophotometer (AAS). Chemical characteristics of collected soils were analysed in the Laboratory of Soil Resource Development Institute (SRDI), Rajshahi and presented in Table 1.

Isolation of Bacteria

Bacteria were isolated by plating onto an agar solidified Minimal Salts (MS) medium. The plates were incubated at 37°C for 4 days and bacterial colonies were found to grow on the medium. Analysis of effluents after release bacteria: dilution (1:3) is shown in Table 2.

Effluent analysis

The samples were prepared by using HNO_3-$HClO_4$ digestion (APHA, 2002). Since the samples were of organic origin with a very high organic content, HNO_3-$HClO_4$ digestion was preferred over the more common HNO_3 extraction for the determination of heavy metals. This strongly oxidizing digestion decomposes organics quickly and efficiently.

To determine the pollution load in these effluents P^H, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), Chromium, Cadmium and Lead concentration was tested. These studies were carried with in 24-48 hrs. after sample collection.

For all the heavy metals analyses of the prepared samples were performed through atomic absorption spectrophotometer. Flame vapor generation technique was used in the determination of heavy metals chromium, cadmium and lead. The physiochemical characteristics of effluents were analysed in the Department of Botany and Water Analysis Center NGO FORUM, Lalmatia, Dhaka and is shown in Table 3.

Effects of effluents on seeds germination

The experiment consisted of five treatments viz. T_1 : Tap water, T_2 : Effluents as such, T_3 : 1:1 dilution, (Effluents : Tap water), T_4 : 1:3 dilution, (Effluents : Tap water) and T_5 : mass culture of isolated bacteria from tannery effluents were released into the diluted effluents (1:3) and small quantity of glucose (0.5mg/ml) were mixed in the diluted effluents and kept for 7 days and the bacterial treatment effluents were used for seeds germination of boro rice (BARI dhan-29), wheat (satabdi) and chickpea (BARI chola-4). Seeds germination was studied by putting the seeds on whatman filter papers placed in sterilized petridishes. The filter papers were well moistened by using equal amounts of the respective waters. Twenty five healthy seeds of wheat, rice and chickpea were placed in each petridish. The petridishes were arranged on a laboratory under room condition. A seed was considered to be germinated when the radical had emerged from the seed coat. The experiment was completed in 7 days. The seedlings were taken out from the petridishes and their respective root and shoot length were measured for each treatment. Control experiment, which was performed with tap water. The data obtained on seeds germination and seedling growth were subjected to statistically analysis using student's t test.

Table 1. Analysis of soil from effluents dumping areas of Hazaribagh.

Parameters	Units	Sample-1	Sample-2	Sample-3	Sample-4	Average	Standard
pH		7.9	7.7	7.8	7.8	7.8 ± 0.08	7.8
Organic mater	%	4.02	4.04	3.88	3.38	3.83 ± 0.30	3.83
Potassium	mhos/100g	0.55	0.82	0.41	0.33	0.52 ± 0.21	0.226-0.30
Calcium	"	23.45	20.25	18.47	11.3	18.36 ±5.14	4.51-6.0
Magnesium	"	2.71	2.82	2.23	0.22	1.99 ±1.21	1.126-1.5
Total Nitrogen	%	0.2	0.2	0.19	0.22	0.20 ±0.01	0.271-0.36
Phosphorous	µg/g	15.7	8.2	10.2	9	10.77 ±3.38	18.1-24.0
Sulpher	"	68.07	77.93	67.37	68.54	70.47 ±4.99	27.1-36.0
Boron	"	0.92	1.09	0.91	0.78	0.92 ±0.12	0.451-0.6
Copper	"	1.18	1.49	0.79	0.75	1.05 ±0.35	0.451-0.6
Iron	"	34.7	26.5	22.6	18.8	25.65 ±6.80	9.1-12.0
Manganese	"	10	11	4.7	4.4	7.52 ±3.46	2.256-3.0
Zinc		9.1	11.32	9.64	8.37	9.60 ±1.25	1.351-1.8

Table 2. Analysis of effluents after release of bacteria: dilution (1:3)

Name of Parameters	Concentration Present (mg/l)	
	Contaminated	Treatments
Cadmium	0.023	0.015
Chromium	93.4	50.5
Lead	0. 74	0.69
COD	2434	650
Dissolved Oxygen	0.8	2.9

Table 3. Analysis of effluents from tanning industry (Monsoon seasons)

Name of Parameters	Concentration Present (mg/l)	Standards for drinking water (mg/l)
Color	Light green	-
pH	5.1	6.5-8.5
Cadmium	<.002	0.005
Chromium	238.6	0.05
Lead	0.36	0.05
(COD) Chemical Oxygen Demand	1421	4
$BOD_{5.20}$ °C	90#/100 ml	0.2
Dissolved Oxygen(DO)	2.0	6
Bicarbonate(HCO_3)	160	-
Total Hardness	240	200-500
Calcium Hardness	147	75
Magnesium Hardness	22.69	30-35
Phosphate Hardness	0.4	6

Table 4. Effects of tanning effluents on seedling growth in different treatments

Treatments	Wheat		Rice		Chickpea	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
T_1	3.02±0.45	1.68±0.39	2.78±0.37	1.57±0.37	3.04±0.98	1.77±0.23
T_2	1.62±0.13	0.8±0.08	2.2±0.46	1.01±0.20	2.65±0.84	1.55±0.09
T_3	1.77±0.32	0.85±0.1	2.41±0.47	1.15±0.19	2.71±0.72	1.57±0.09
T_4	1.86±0.90	0.8±0.16	2.72±0.36	1.51±0.36	2.87±0.86	1.68±0.17
T_5	2.55±1.62	1.33±1.04	2.75±0.37	1.53±0.35	2.84±0.65	1.72±0.21

T_1= Tap water (control); T_2=Effluents as such; T_3= 1:1 dilution; T_4=1:3 dilution; T_5=1:3 dilution, treatment by bacteria.

Values followed by the same letters in the same column are not significantly different at $p<0.05$ ± standard error of the mean.

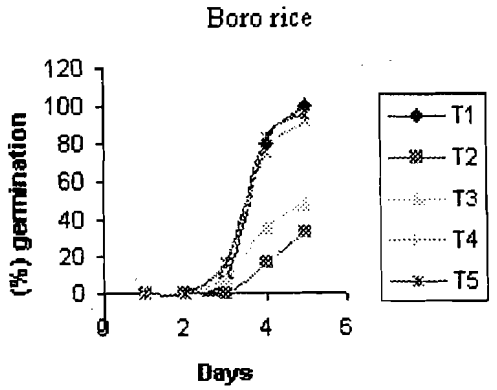

Fig. 1

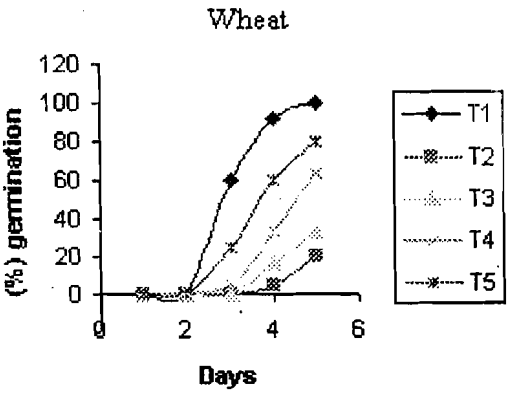

Fig. 2

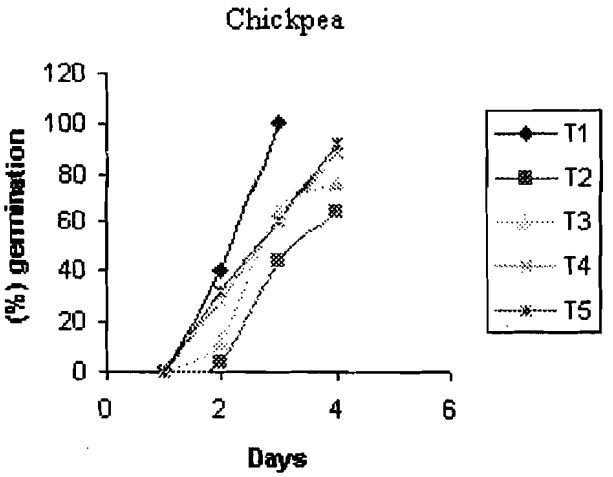

Fig. 3

Fig.1-3. Percentage of germination of seeds of Boro rice, Wheat and Chickpea in different treatments of tannery effluents

Results

Analysis of soil

Heavy metals like Zn, Cu, Mn, Fe, Co were detected in the soil sample (Table 1). The percentage of heavy metals were similar with that of cultivated soil. Total nitrogen content was moderate but the percentage of boron, calcium, sulpher, iron, manganese and zinc were very high. Due to imbalance of heavy metals no cultivation was found around the 10 km. of dumping areas of effluents at Hazaribagh.

Physico-chemical characters and metals in effluents

The physico chemical characteristics of effluents are presented in Table (3). Effluents colour is light green and its smell like rotten egg.

pH of the experimental effluent is 5.1. Total hardness, calcium, magnesium and phosphate hardness values are 240, 147, 22.69 and 0.4 mg/L, respectively. The COD value is 1421. Chromium, lead, bi-carbonate values are 238.6, 0.36 and 160. Remaining metals like chromium, lead and cadmium values are compared to standards, not suitable for irrigation.

Seed germination and seedling growth

Figures 1-3 shows changes with time in percentage germination of boro rice (*O. sativa)*, wheat (*T. aestivum*) and chickpea (*C. arietinum*), respectively. The rate of seed germination of wheat and rice were lower in T_2 (effluents as such) and T_3 (1:1 dilution) as compared to T_1 (Tap water) and T_4 (1:3 dilution), while in case of chickpea, the rate of seed germination was initially faster in treatments T_1 (Tap water) and T_2 (Effluents as such) then those in other treatments i.e., T_3 (1:1 dilution) and T_4 (1:3 dilution).

In seedling growth of the test crops, root and shoot length were not significantly affected by the five treatments in either of the species (Table 4). The results indicate the inhibitory effects of effluents on seed germination as well as seedling growth on the test crops. But after treatment of the effluents by bacteria, the seeds germination was relatively better as compared to effluents as such. On germination wheat remained more sensitive and susceptible to toxic effluents but chickpea proved to be more tolerant. The effluents from leather tannery are not suitable for agricultural use but after treatment with bacteria, no deleterious effect was found in the germination of seeds. It can be inferred that released bacteria are able to detoxify the toxic pollutants (Table 2). The chemical oxygen demand (COD) and concentration of chromium and cadmium decreases in the bacteria treated effluents. The initial COD value was 2434 mg/l but after release of bacteria in the same effluents it decreases up to 650 mg/l. In the same time the dissolved oxygen (DO) increased in the diluted effluents (Table 2).

Discussion

Irrigation water quality must be considered not only with regards to its immediate effect on soils and crops but also with regards to the welfare of consumers. Pesticides, pathogens and even some naturally occurring water constituents may not affect the crops directly but may affect animals or human beings and so are equally important criteria of water quality (Rhoades and Bernstein, 1971). It was indicated above that the waste water of the industrial plant is not being used directly for purposes of irrigation. Instead it was the canal water, into which the tannery waste water effluent is being discharged, that is being used for irrigation purpose. Ghimire and Bajracharya (1996) reported significant differences in the sensitivity of four type of vegetable seeds (*Brassica juncea, B. oleracea, B. rapa* and *Raphanus sativus* of a single family Brassicaceae) to the effluents of tannery, carpet dyeing and steel industry.

Gupta and Chapagain (1999) observed significantly different effect of polluted water of Dhobikhola on *Pisum sativum* and *Lepidium sativum*. Jha and Niroula (1998) showed differential sensitivity to various industrial effluents and municipal sewage on germination and dry weight increases on rice and blackgram. In this study also, rice and wheat showed differential sensitivity to the tested effluents on germination and seedling growth. Sharma and Aery (2000) found that low concentration of heavy metal chromium was stimulatory to the plant growth while other workers contradicted it and observed that chromium is inhibitory and toxic to plant growth. Chromium toxicity manifested itself in plants by inhibiting the growth more or less, showing

chlorosis with small brownish-red or purple leaves and necrotic lesions. High chromium concentration inhibits photosynthesis and greatly inhibits the root growth. It is, therefore, evident that chromium affects the plant growth mainly by damaging the root while its translocation into other parts of the plant is of minor importance. Reduction in germination and seedling growth in the test crops by the industrial effluents in the present study may be due to heavy metal toxicity inhibiting the functions of essential enzymes (Jerome and Ferguson, 1972).

Findings of the present study lead to conclude that the seeds germination of rice (*O. sativa*), wheat (*T. aestivum*) and chickpea (*C. arietinum*) are seriously affected by the tannery effluents, even after dilution of the effluents by fresh water. But after treatment of the effluents with bacteria, the seeds germinate smoothly like control seeds. The released bacteria are able to degrade or detoxify the toxic pollutants.

Acknowledgemens

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EFFECT OF ALCOHOLIC BEVERAGE 'MAHUA' ON MALE REPRODUCTIVE INDICES OF ADULT ALBINO RATS

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Abstract ■ "Mahua", an alcoholic beverage contains 30% to 40% of alcohol. It is widely consumed by tribal people particularly in western part of West Bengal. This beverage is produced by fermentation of jaggery with petal of "mahua" plant. Chronic ethanol consumption causes sexual dysfunction and affects hypothalamic-pituitary gonadal axis. The aim of this study was to find out effect of "mahua" consumption on male reproductive system. The experiments were carried out on male albino rats. Animals were divided into three groups: one control and two experimental groups. First experimental group of animals were treated with "mahua" for 30 days and second experimental group for 60 days. In our present investigation treatment of rat with "mahua" significantly reduced testicular weight, sperm count, sperm motility and sperm viability. Sperm morphology is also adversely affected by "mahua" treatment. The testicular protein and endogenous antioxidant level is reduced where as cholesterol level is increased in 'mahua' treated rats. The results of this study suggest that "mahua" treatment adversely affect male reproductive indices and such effect may be due to oxidative stress.

Key wards: Alcohol, "mahua", sperm count, sperm motility, sperm viability,

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Introduction

Madhuca indica commonly known as "mahua" is an Indian tropical tree found in the central and North plains. 'Mahua' flower is edible and a food item for tribal. It is also fermented to produce alcoholic drink, and is also known as 'mahua'. It is used by a number of people as a country liquor. It is widely consumed by tribal men and women during celebration. This beverage is produced by fermentation of jaggery with petal of "mahua" plant (*Modhuca indica*).

Alcoholic beverages are found to affect different systems of the human body, including reproductive system. The testes has been shown to be highly susceptible to ethanol as it cross the blood testes barrier (Maneesh et al, 2005). Chronic ethanol consumption causes sexual dysfunction (Van Thiel and Lester, 1997). It negatively impacts all level of hypothalamic pituitary gonadal axis (Emanuel and Emanuel, 2001). Alcohol itself is the principal cause of hypogonadism (Bannister et al. , 1987). Early histological studies indicate that testis may be even more sensitive to ethanol than the liver (Arlitt and Wells, 1997). Chronic alcoholics are often associated with impotence, loss of libido, premature or delayed ejaculation and sterility (Bayden and Pamenter, 1983). Alcoholics have been shown to increase number of teratozoospermia (Villalta et al., 1997). Oxidation of alcohol, a process that occurs as a part of alcohol metaboiism generates bi-products call oxidants that can contribute cell damage(Maneesh et. al., 2005). ROS causes damage of tissue protein, carbohydrate and DNA (Agarwal and Prabakaran, 2005). Alcohol exposure causes oxidative stress from imbalance between ROS formation and their efficient removal by available antioxidant system (Turner and Lysiak, 2008).

The present study was undertaken to assess the effect of "mahua" on male reproductive indices and its relation with testicular antioxidant level.

Material and Methods

Animals and grouping

The study was approved by institutional ethical committee and the animals were maintained and sacrificed following the standard ethical procedures. Pure wister strain male albino rat of 5-6 month age having body weight between 140- 180 gm were used for our experiments. The animals were housed in standard laboratory condition in a photoperiod cycle of 12 hr : 12 hr (light and dark) and were supplied with standard laboratory diet and drinking water *ad libitum*. Animals were randomly divided into three groups- one control and two experimental group. Each group contains 6 animals. Animals of both the experimental group were orally treated with "mahua" (25 mg /kg body weight /day) for 30 days (1st group) and 60 days (2nd group). Dose was selected according to Srikanth et al. (1999). Animals of control group were orally given distilled every day for 60 days as placebo treatment.

Separation of epididymal sperm :

Epididymal spermatozoa were separated by modification of method of Brooks (1976). Caudal portion of epididymis was cut out . It was then cut in to small pieces by sharp blade. Spermatozoa from epididymal pieces were removed by votexing gently in Krebs Ringer phosphate buffer (pH 7.4) for 10 min . Suspension was used for sperm count .

Sperm concentration :

Spermatozoa were counted as per the method of Zaneveld and Polakoski(1977). Sperm suspension was placed on both side of Neubaure's hemocytometer and allowed to settle for 15 min. The number of spermatozoa in the appropriate squares of the hemocytometer

was counted under the microscope at 40x magnification. The sperm concentration was expressed as number of sperm per cauda of epididymis as well as number of sperm / gm weight of epididymis.

Sperm viability:

Assessment of viability of sperm was done by hypoosmotic swelling test (Buckell, 2003). 0.1ml epididymal sperm extract was mixed with 1.0ml 150m mol/kg hypoosmotic solution (prepared by 7.35 gm sodium citrate and 13.5 gm fructose in 1000ml distilled water). The mixture was incubated 60 min at 37° C. Then 0.2ml of the mixture was placed on a slide and mounted with a cover slip and immediately examined at a magnification of 400. The percentage of reacted sperm (curled tail) and unreacted sperm (uncurled tail) were assessed by counting 100 sperm.

Sperm motility

For the study of sperm motility, spermatozoa were expressed out by cutting the distal end of cauda epididymal tubule (Bagchi , 1998). It was then diluted with physiological saline and placed on a thin glass slide. The sperm motility was studied according to the method of Aboua et.al., (2009). Ten random fields were manually scored for the number of motile and non-motile sperms. Motility was expressed as a percentage of motile sperm compared to total sperm counted.

Sperm morphology

For the study of sperm morphology, spermatozoa were expressed out by cutting the distal end of cauda of Epididymal tubule (Srikanth et al, 1999) . Spermatozoa with Epididymal fluid was diluted with physiological saline and a smear was prepared . It was then air dried and fixed in a mixture of equal part of ethanol and ether. The slides were then stained with Basic Fuschin. The slides were then examined under oil immersion objective (Dada et. al. , 2001). Morphologically normal and abnormal sperm were then counted (Pramanik and Mondal, 2010). Abnormal sperms were classified according to their morphology : I) small head, ii) double head or triple head , iii) coil tail and iv) short thick tail (Dada et. al . ,2001).

Biochemical estimation of testicular protein, SOD and vitamin-C :

Testis were removed and immediately placed in cooled 0.9% NaCl and washed in the same (Sisodia et al , 2008) and homogenized in 0.9% sodium chloride solution (1 ml per 50 mg tissue). Suspension was allowed to settle for 15 min at -20°C. Supernatant was centrifuged at 800 x g for 15 min (Srikanth et al , 1999) and used for estimation of protein, SOD and vitamin C following the method of Lowry (Das and Dasgupta , 2002), Martin et al (1987) and Srikrishna and Suresh (2009). The result was expressed as mg/gm weight of testis.

Biochemical estimation of testicular cholesterol

Testis was homogenized in ether-alcohol mixture (1ml/50mg tissue). Supernatant was used for estimation of cholesterol by ferric chloride method (Nath and Nath, 1990). The result was expressed as mg/gm weight of testis.

Statistical analysis : The data were expressed as mean ± standard error of mean and analyzed for significant differences by one-way analysis of variance. Value of $p < 0.05$ was considered statistically significant.

Results

Treatment of male albino rat with alcoholic beverage 'mahua' significantly decreased testicular weight in duration dependent manner (Table-1). The results of ANOVA showed significant results ($p<0.001$). The multiple comparison test revealed that there were

Table 1: Effects of 'mahua' treatment on percentage contribution of testicular weight on body weight

Animal group	Percentage of testicular weight in respect to body weight	p-value
Control	1.35 ± 0.07	
30 days 'mahua' treated	1.02 ± 0.05	<0.001
60 days 'mahua' treated	0.86 ± 0.05	<0.001

Data represent mean ± SD (n=6 in each group)

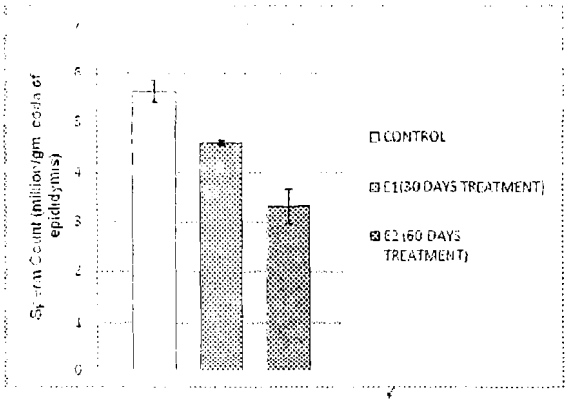

Fig.1a: Effect of 'mahua' treatment on epididymal sperm concentration (per cauda). Bars and vertical lines represent mean value (n=6) and SD respectively. (In treatment groups P<0.001 w. r. t. control group)

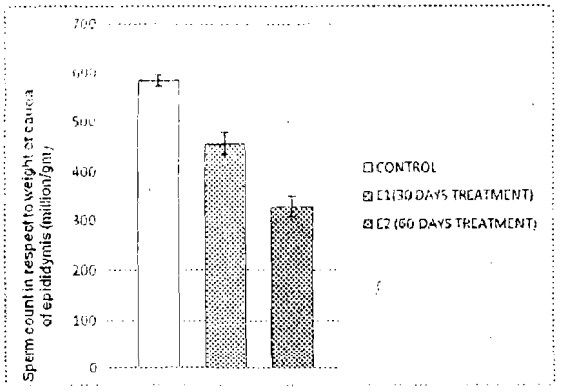

Fig.1b: Effect of 'mahua' treatment on epididymal sperm concentration (per gram weight). Bars and vertical lines represent mean value (n=6) and SD respectively. (In treatment groups P<0.001 w. r. t. control group)

significant differences between control and 30 days treated groups (p<0.001), control and 60 days treated groups (p<0.001), and 30 days treated and 60 days treated groups (p<0.001). Oral administration of 'mahua' decreased sperm concentration in each cauda of epididymis (fig.1a) as well as per unit weight of cauda (fig.1b). The percentage of reduction was more in 60 days treatment than that of 30 days treatment (Fig.1). There was negative correlation between 'mahua' treatment and sperm count (r= -0.8).

Treatment of male albino rat with "mahua" affected the sperm viability. The percentage of viable sperm in experimental groups of rats was lower than that of the control group (Fig.2). Similar result was obtained for sperm motility (Fig.3).

The treatment of "mahua" on male albino showed alteration in sperm morphology (Table-2). The percentage of abnormal sperm in experimental groups was higher than the control group. Morphologically abnormal sperms with coiled tail, short thick tail and defective head were higher in experimental groups than that of the control group.

Figure- 4 shows the effects of "mahua" treatment on testicular protein level. The testicular protein level was decreased significantly in animals those are treated for 30

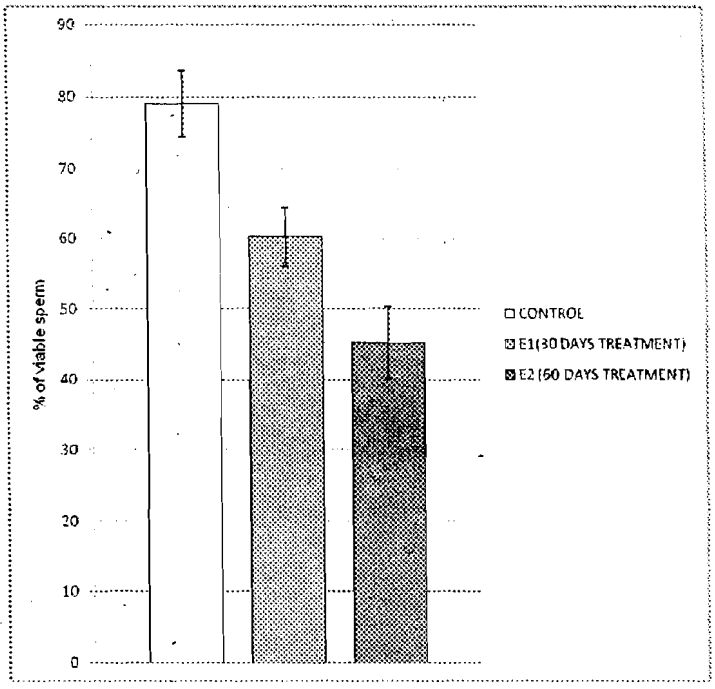

Fig.2: Effect of 'mahua' treatment on viability of epididymal sperm. Bars and vertical lines represent mean value (n=6) and SD respectively. (In treatment groups $P<0.001$ w. r. t. control group)

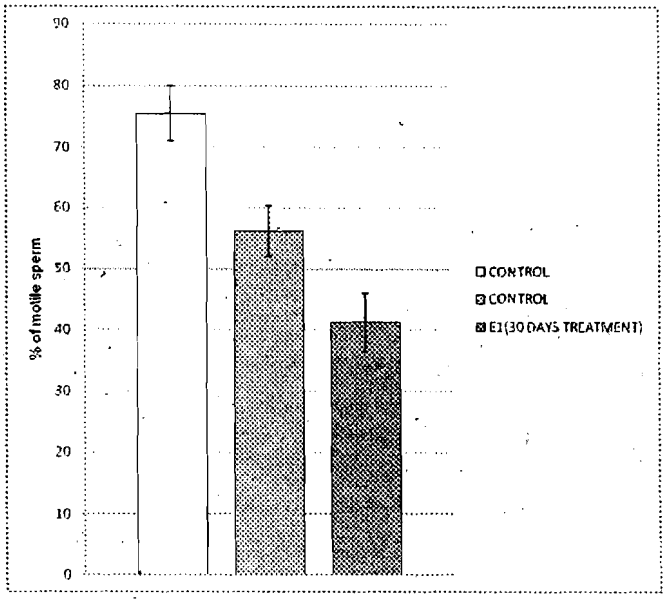

Fig.3: Effect of 'mahua' treatment on motility of epididymal sperm. Bars and vertical lines represent mean value (n=6) and SD respectively. (In treatment groups $P<0.001$ w. r. t. control group)

Table-2. Effect of 'mahua' treatment on sperm morphology

Animal group	Normal sperm (%)	Abnormal sperm (%)			
		Defective head	Short and thick tail	Curled tail	
Control	88.9	2.2	5.3	3.6	11.1
30 days 'mahua' treated	66.4	12.2	15.3	6.1	33.6
60 days 'mahua' treated	55.0	18.4	17.3	9.3	45

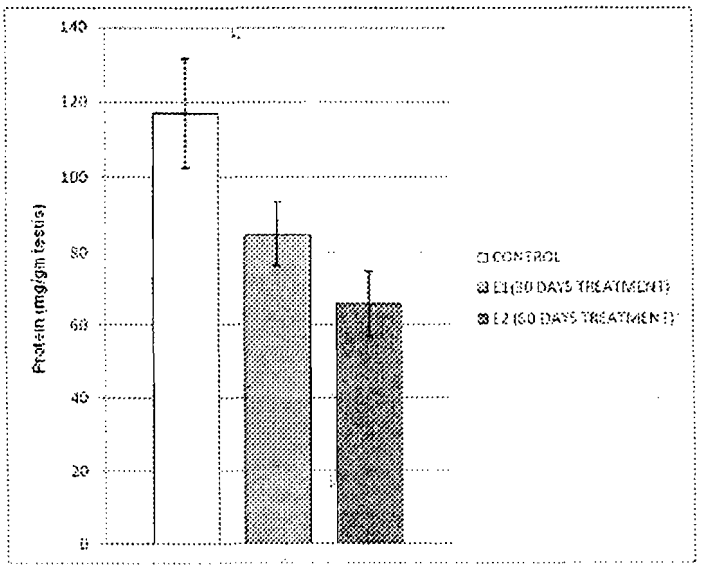

Fig.4: Effect of 'mahua' treatment on testicular protein level. Bars and vertical lines represent mean value (n=6) and SD respectively (In treatment groups P<0.001 w. r. t. control group)

days (p<0.001) as well as 60 days (p<0.001). It was noted from the results that the administration of "mahua" significantly increased (p<0.001) testicular cholesterol level. The quantity of increase was greater in 60 days treatment group than that of 30 days treatment group (Fig.5).

The SOD , the principal antioxidant enzyme of testis was estimated in control and 'mahua' treated albino rats. The results showed that 'mahua' treatment significantly decreased (p<0.001) the activity of SOD in testis (Table - 3). Both the treated group showed significant decrease in SOD in comparison to control animals.

The Vitamin -C , the one of the nonenzymic antioxidant of testis was estimated in control and 'mahua' treated albino rats. The Vitamin-C level was significantly decreased in testis (p<0.001) in 'mahua' treated groups than that of control group. The effect was greater in 60 days treatment group than that of 30 days treatment group (Table 4).

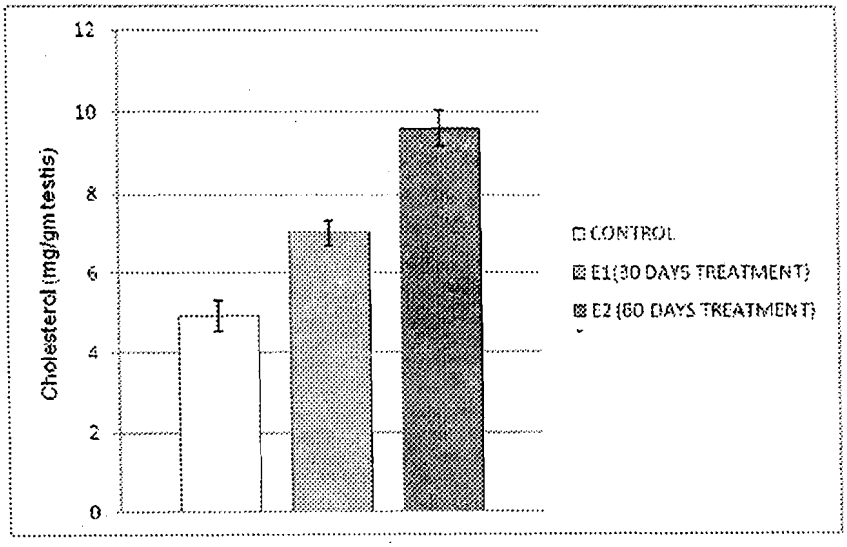

Fig.5: Effect of 'mahua' treatment on testicular cholesterol level. Bars and vertical lines represent mean value (n=6) and SD respectively (In treatment groups $P<0.001$ w. r. t. control group)

Table. 3: Effect of 'mahua' treatment on the activity of super oxide dismutase of testicular tissue

Animal group	Super oxide dismutase (U/mg tissue)	p-value
Control	0.528 ± 0.076	
60 Days treatment	0.385 ± 0.066	0.001
90 Days treatment	0.243 ± 0.089	0.001

Data represent mean ± SD; n=6 in each group

Table. 4: Effect of 'mahua' treatment on the level of ascorbic acid in testicular tissue

Animal group	Ascorbic acid (ug/ mg tissues)	p-value
Control	11.40 ± 1.428	
60 Days treatment	7.74 ± 0.810	0.001
90 Days treatment	5.49 ± 0.506	0.001

Data represent mean ± SD; n=6 in each group

Discussion

The treatment of 'mahua' significantly decreased weight of testis in duration dependent manner (Table1). This result supports previous observation of Adler (1992). Decreased in testicular weight is coincide with reduced protein content in it (Fig.5).

The reduced sperm content in cauda epididymis of 'mohua' treated rats implies an adverse effect of 'mahua' on spermatogenesis. This effect of 'mahua' may be due to alcohol, which is the main constituent of this liquor. A number of clinical and experimental studies have shown impaired spermatogenesis under chronic ethanol consumption (Willis et al, 1983; Haider et al.,1985). Anderson et al (1983) also observed low sperm count content in cauda of epididymis of ethanol consumed rats. Ethanol-treatment decreases testosterone level in serum, testis as well as epididymis (Adam et al, 1991). Alcohol impairs testosterone production and causes testicular atrophy (Maneesh, 2006). Increased oxidative stress is a well accepted mechanism of alcohol-induced injury of various tissues including testis (Emanuele et al, 2001). Low testosterone is due to increase of oxidative stress which can damage testosterone secreting Leydig cell and supporting cells. Alcohol administration reduced activity of enzymes in testis crucial to the synthesis of steroid sex hormones (Chiao and VanThiel, 1983). Significantly increased cholesterol level due to 'mahua' treatment (Fig. 5) indirectly supports that 'mahua' induces the decrease of cholesterol utilization for testosterone synthesis. A number of androgen regulated proteins secreted from seminiferous tubule are essential for spermatogenesis (Sharpe et al, 1992). The 'mahua'-induced decrease of testicular protein levels may be a cause of low sperm content in cauda epididymis.

Oxidative stress has been established as one of the cause of male infertility (Makker et al,2009). Oxidative stress is detrimental to cell function and survival (De Lamirande and Gagnon, 1995). In mammals the epididymis plays an important role in the maturation and storage of sperm. During epididymal transit, sperm metabolism increases, accompanied by the threat of oxidative stress (Dacheux et al,2003). Oxidative stress is a cellular condition associated with an imbalance between the production of ROS and their scavenging capacity by antioxidants. When the production of ROS exceeds the available antioxidant defense, significant oxidative damage occurs to many cellular organelles due to damage of lipids, proteins, carbohydrates and DNA. These processes can ultimately lead to cell death. Sperm is susceptible to oxidative damage as it content high poly unsaturated fatty acid in its plasma membrane (Sanoke and Kurpisz, 2004). Though antioxidant defense system is active in the semen its activity is limited as the amount of cytoplasm of the sperm is low (Lewis et al,1997). Leukocytes and spermatozoa have been shown to be the main sources of ROS (Garrido et al,2004).We studied the activity of SOD, a principal enzymatic antioxidant of seminal plasma and the level of vitamin C , a nonenzymatic antioxidant. Activity of SOD was found to be significantly lower in 'mahua' treated rats than that of control rats (Table-3). Similarly, the ascorbic acid level was also significantly lower in the animals of 'mahua' treated groups in comparison to that of the animals of control group (Table-4). Thus, the results indicate that 'mahua' treatment induces oxidative stress.

The sperm motility was found to be decreased due to the treatment of 'mahua'. Impaired motility of human spermatozoa was also noted in chronic ethanol users (Kucheria et al,1985; Gamathi et al, 1993). 'Mahua'-induced

decrease in sperm motility might be due to decrease of ROS scavenging capacity. Previous studies have shown a correlation between high level of ROS and sperm motility (Agarwal et al, 2003). The ROS, particularly H_2O_2 might diffuse across the membrane into the cells and inhibit the activity of glucose -6-phosphate dehydrogenase which lead to a decrease in the availability of NADPH. As a result there is decrease formation of ATP which is essential for sperm motility (Aboua et al,2009).

In conclusion, 'mahua', an alcoholic beverage, affect male gonads by decreasing testicular weight, sperm content in epididymis, sperm motility and sperm viability in rats. Such adverse effect may be due to increased oxidative stress.

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MANAGEMENT PROBLEMS AND STRATEGIES OF GRASSLANDS IN JALDAPARA WILDLIFE SANCTUARY, WEST BENGAL, INDIA

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Abstract ■ The Sanctuary is situated in the foothills of Eastern Himalaya, in the civil district of Jalpaiguri, West Bengal, comprising of 12 forest blocks and 46 compartments. As it is situated in the flood plains of river Torsa and its tributaries containing savannah grasslands which harbors 72 Great Indian One Horned Rhino. Besides Rhino, JWLS contain other 15 species of animals belong to schedule – I of Wildlife (Protection) Act, 1972. The shape of the sanctuary is trouser like in the southern part having maximum wild animal concentration. There are 32 revenue villages, 4 forest villages, and 9 tea gardens situated in and outside the sanctuary having approximately 9,000 human and 10,000 cattle population which create tremendous biological pressure on the sanctuary. Wild herbivores of JWLS share the Grasslands with domestic livestock of fringe villages which gradually deplete the quality and quantity of forage for grazing as well as cause destruction of habitat. Grasslands of JWLS are in seral stages of natural riverine succession so it has a tendency to become woodlands gradually. To protect the grasslands some managerial intervention is necessary through proper scientific strategies which will maintain the quality of grasslands, provide food to the wild herbivores without much damaging the natural habitat. Some suggestions have been put forth in this paper.

Introduction

Jaldapara Wildlife Sanctuary (latitude 25°58' to 27°45' North and longitude 89°08' to 89°55' East) hereafter Sanctuary, is located in the foothills of Eastern Himalayas in the Jalpaiguri District of Northern part of West Bengal, India. It was declared as a game sanctuary in 1941 comprising an area of 99.50 sq.km, with two subsequent extensions in 1976 and 1990, the area was increased to 216.50 sq. km. covering 12 forest blocks and 46 compartments. The Sanctuary is trouser shaped in the southern side with 12 revenue villages all along the boundaries with human population of 90,000 and about 1,000,000 livestock.

The sanctuary serves as a gene pool for the great Indian one horned rhinoceros (*Rhinoceros unicornis* L), an endangered species. It represents Bio-geographic zone 7 B (Lower Gangetic Plain) as suggested by Rodgers and Panwar (1988). The sanctuary consists of 16 species of vertebrates (Pandit, 2004; Pandit *et al., 2004;* Pandit & Sinha, 2006) belonging to Schedule-I of Wildlife (Protection) Act of 1972 (Anonymous, 1994). These Indian One Horned Rhinoceros, Royal Bengal Tiger, Indian Gaur, Asian Elephant, Sloth bear, Leopard, Hog badger, Swamp deer, Hispid hare, Bengal florican, Python, Pangolin, Peafowl, Indian pied hornbill and Large falcon are the star attraction of the area. The sanctuary has 33 species of mammals, 230 species of birds, 24 species of reptiles, 30

species of fishes (Pandit, 1996, Pandit & Yadav, 1996, Pandit, 2004, Pandit & Sinha, 2006). Jaldapara is also rich in floral diversity and it houses 384 species of flowering plants (Banerjee, 1993) belonging to 429 genera under 111 families. Out of them, 19 are orchid species and 71 are grass species including 47 threatened species belonging to 29 genera. Recently Das *et al.*, (2003) have reported 224 species (141 dicotyledonous, 51 monocotyledonous and 32 pteridophytes) of flora of the sanctuary which are palatable to the local fauna.

Endangered Toto Tribe having a total population of 1000 people only (Pandit, 2006; Pandit, 2004; Pandit & Sinha, 2006) resides in Toto Para, located in the northern hilly part of the sanctuary near Titi Block. The tribe's existence and preservation of culture is also of major concern. Today Jaldapara has excellent potential for tourism. Every year it attracts good numbers of visitors through which local people get direct and indirect benefits.

Out of 32 villages, 11 villages are situated in between two legs of the sanctuary. The width of the leg varies from less than 1 km to 4.5 km (Pandit, 1996). Villagers are poor, illiterate and mostly belong to scheduled caste and tribes and are agriculturally backward with little alternative hence highly dependent on the forest based resources for their livelihood which exerts huge pressure on the sanctuary. Moreover, 9 tea gardens are situated around the sanctuary and its labourers exploit the natural resources of it for their domestic needs. Besides, River Torsa, other perennial water sources of the sanctuary are Hollong, Sissamara, Buritorsa, Chirakhawa, Bhaluka, Sanjoy, Gorumara, Bania, Sukti, Sukta and Malangijhora etc...which provide water throughout the year to the wild animals (Pandit, 1996, 2004).

Floral types

Major floral types of the Sanctuary are (i) Riverine forest (ii) Wet mixed forest (iii) Sal forest (iv) Semi evergreen forest (v) Evergreen forest (vi) Savannah grassland (Moist sal savannah; Low alluvium savannah woodland; Eastern Alluvium grassland; Primary grassland) and (vii) Hydrophytic vegetations (Banerjee, 1993; Anon, 1997; WII, 1997; Das *et al.*, 2003, Pandit *et al.*, 2004).

The grasslands of this area are in seral stage of natural succession and tend to be replaced by forest. Due to this a trend of invasion of tree species in grassland with seedlings (1000-2000 per ha.), saplings (500-1000) and trees (50-150 per hec.) of *Dalbergia sissoo* (sissoo), *Bombax ceiba* (simul), *Accacia catechu* (khair), *Albizia procera* (siris), *Lagerstroemia parviflora* (sidha), *Dillenia pentagyna* (tantari) etc. are conspicuous (Pandit,1996 &2004). The grasslands in the flood plain area are naturally maintained by river course. The river Torsa has changed its course in 1968 flood from west to east and most of the erstwhile grasslands are dry. Trees are emerging and covering the grassland and some areas have already been covered with them.

Pure grassland, grassland with khair – sissoo and simul – siris succession which cover approximately 20% of the total area of sanctuary (Pandit *et al.*, 2004) are most important habitat of Rhino, Hogdeer, Barking deer and other herbivores. Present population of Rhino as per 2002 census is 72 (Pandit, 2004).

Problems in the Grasslands

1. ***Grazing by livestock from Fringe area villages***

Approximately 1,00,000 livestock from 32 fringe villages, 4 forest villages and 9 tea gardens are grazing inside the Sanctuary area. On estimation by laying out sample plots (Pandit, 1996, 2004) it was found that at least 11,000 cattle graze inside the sanctuary which is one of the prime grassland area for rhino, elephant and hog deer. The intensity of cattle grazing varies from block to

block. Grazing of livestock inside the sanctuary depletes the fodder of wild herbivores and transmits diseases like Foot and Mouth Disease, Anthrax, Rinderpest to wild animals.

2. *Weeds and Climber infestation*

Climbers like *Mikania sps.* and weeds like *Leea sp, Lantana sp, Eupatorium sp* and *Cassia tora and ferns* are not only damaging but encroaching the prime habitat of Rhino and other wild animals.

3. *Encroachment of woodland in grasslands*

Succession of tree species is one of the most important factors for change in the grassland composition of Sanctuary. Another factor which is operating in the existing climatic conditions is the fertile soil and under ground water regime. Grasslands are colonized by khair-sissoo, simul-siris association as a riverine succession which is gradually converting the major grassland areas in to woodland that ultimately is leading to a decrease in the prime rhino habitat.

Table – 1: Number of trees according to age class.

Age class	Number of trees
0.0 - 2.0 years	1757
2.0 - 4.0 years	503
4.0 – 6.0 years	109
6.0 - 8.0 years	605
8.0 - 10.0 years	18
10.0 - – 12.0 years	-
12.0 - 14.0 years	
Total number	2992

To assess the number of tree species in the grassland few sample plots were laid down randomly in the grassland area encroached by woodland species. Tree species were counted from seedling stage. It was to fully grown stage and found that not only species like Khair, Sissoo, Simul, Siris; other associated species also have started encroaching in this area. Other species are *Lagerstroemia parviflora* (Sidha), *Dillenia pentagyna* (Tantari), *Careya arborea* (Kumbhi), *Syzigium cumini* (Jamun), *Sterculia villosa* (Udal), *Trewia nudiflora* (Pitali), *Bischofia javanica* (Kainjal). 2992 trees per hectare were found (Table- 1). If we assume that 40 percent of these trees are in well established stage their number is around 1200 per hectare.

It has been found that in JP-3 compartment *Sidha* is predominant, while in Malangi-I, *Tanatri* is predominant. *Khair-sissoo, Simul - siris* association colonizes grassland of JP-1, 2, 3, 4 and Haashimara-3, 4 compartments. These successional stages are drastically reducing the grassland area which is a major threat to the endemic wildlife communities including Rhino, Hog deer, Hispid hare, Bengal florican etc. . These grassland areas are a prime rhino habitat and regularly used by the rhinos. Maintaining the present composition of grassland and woodland is therefore very important from management point of view.

4. Fire

Grassland fire in the Sanctuary occurs every year although not in great extent. Unfortunately no regular records on map are kept in respective ranges, but such information is very important from management point of view. Fire in grassland is mainly caused by illicit grazier, illegal collector of simul floss (*Bombax* pods), thatch and other forest produce. On many occasions poachers in an attempt to divert the attention of staff lit the grassland area and took the opportunity of poaching in another area. Systematic, judicious, well planned, year wise grassland burning in mosaic manner is a management tool and gives a better result in the management of grassland. It is one of the cheapest management strategies which provides fresh forage in short span of time.

5. *Past Forestry Practices*

Management of forest plays a significant role in maintaining original habitat or vegetation. Therefore, human intervention in terms of management inputs is required to maintain desirable habitat. Species, which are preferred by the existing animals, should be considered for plantation in the protected areas. Unfortunately, it has not been considered in the earlier days and only commercial timber trees were planted in the grassland area and other prime habitats of wild animals. Since grassland area was considered as a wasteland, mono crop like teak (*Tectona grandis*), *Sidha* and other tree species were planted which are not preferred by wild herbivores in some areas. These areas are excellent habitat of wild animals especially for the wild elephants.

Management Strategies

To maintain the grassland in Jaldapara Wildlife Sanctuary management intervention is required as per the need and its timely implication in current situation when human presence and resource is common. Therefore, if nature allows taking its own course then through natural riverine succession process grassland will be converted to woodland. So habitat improvement work is important based on following principles.

A. Preservation and maintenance of the diversity of habitat.

B. Protecting the habitat against the factors causing degradation.

C. Selection of species should be area specific considering animal preference in holistic pattern.

D. Maintenance of habitat in such a way, that not only provides resources to target species but also to the associated species.

Currently following management inputs are needed for the maintenance of existing grassland in Jaldapara Wildlife Sanctuary.

1. *Overhead removal and fodder plantation*

As a riverine succession different woody species appear in savannah grassland of JWLS so for the betterment of the wild animals management inputs is necessary by removing tree species systematically to open the canopy followed by plantation of indigenous tall and short grasses along with local fruit species for the wild animals. It should be done on the basis of the following guidelines.

i) No large gap should be created during removal of big trees.

ii) At least 100 trees per hectare should be retained.

iii) Only indigenous tall and short grass species, local fruit trees and bamboo should be planted.

iv) People should be educated to understand that overhead removal is part of wildlife management and it is not a clear felling of trees. Otherwise local people will take this step in other way and initiate a mass demonstration.

2. *Plantation of indigenous species*

After selection of the suitable area, uprooting or cutting of woody tree species from grassland should be carried out by retaining 100 genetically superior trees per hectare as shade and cover to wild animals. Then repeated cleaning / burning should be done followed by ploughing to uproot the weeds and climbers. The area should be then kept open for sun dry. Soil of proposed plantation area must be tested for pH, organic carbon, organic matter, total nitrogen, available phosphate, potassium etc.

Fodder plantation should be completed within June. Tall fodder grasses recommended are *Saccharum bengalense, S. arundinacea, S. fuscum, Arundo donax, Pharagmitis karka, Coix lachrymajobi, Setaria spp. Themeda villosa* and fruit species will be Jack fruit, *Terminalia chebula, T. belerica, Artocarpus chaplasa, Dillenia indica ,D. pentaphylla, Emblica officianalis and Alpinia spp.*

Spacing of plantation should be 1.0m × 1.0m.

Subsequent maintenance by weeding cleaning and beating up in blank areas should be done in thrice for the first and second year and twice for third year. Older fodder plantation should also be maintained as per above schedule.

Area should be selected as per priority basis and the areas within 0.5 km. of fringe village should not be planted. All the works should be done with the help of Eco-development Committee members. Short fodder grasses like *Cynodon spp., Panicum spp., Chrysopogon spp., Paspalum spp. and Vetiveria spp.* should be preferred as small patches in between tall fodder grass in 4:1 ratio.

3. *Eradication of weeds and climbers*

Eradication of weeds like *Lantana camara, Leea spp., Eupatorium spp. Cassia tora and Clerodendron spp.*, should be done manually by uprooting. Climbers like *Mikania spp.* should be cut at ground during October and *Leea spp.* must be cut during September

4. *Eradication of Thatch grass and Cymbopogon species*

Heavy thatch grass and *Cymbopogon spp.* growth in JP-2,4,5 and Torsa-2&3 compartments are a problem because wild animals do not prefer these grasses very much. Rhino, elephant and other herbivores sometime eat only inflorescence stick of *Cymbopogon spp.* Thatch grasses when young only eaten by herbivores. Moreover, these grasses cause fire hazard. These species should be eradicated followed by plantation of suitable indigenous fodder species. Thatch grass should also be allowed to cut as per E.D.C. (Ecodevelopment Committee) resolutions in a control way.

5. *Judicious use of fires*

Control burning of natural and fodder plantation areas plays significant role for production of nutritive and palatable fodder to wild herbivores. It has been found that grasses become coarser and unpalatable with increasing age and unpalatable tall grasses are prone to fire. It has also been found that if tall unpalatable grasses are cut and control burnt during cool period rhino and other herbivores utilize these areas more frequently.

Following guidelines are important for control burning

(i) Burning should be done during December and January.

(ii) It should not be done on very windy days when fire can spread in large area.

(iii) Ensure that wild animals are not in trapped by fires.

(iv) Burning should be done in small areas at a time.

(v) Cutting followed by burning should be done in fodder plantation area.

(vi) Monitoring of burnt area and records of all fires should be kept properly for developing future management strategies.

(vii) Burning regime should be two year interval for both natural and well as planted grassland in dry areas and 3 year interval in moist areas. Further evaluation and research is necessary to decide the burning regime in future.

Besides Block wise burning, other small pockets damaged by wildlife or other reasons should also be burnt under close supervision.

Table – 2: Burning regime and schedule

	Moist grassland			Dry grassland		
FB - 1	1st	4th	7th	1st	3rd	5th
FB - 2	2nd	5th	8th	2nd	4th	6h
FB - 3	3rd	6th	9th	3rd	5th	7th

Burning schedule should be prepared first and not all the areas should not be burnt at a time (Table - 2). Each moist grassland areas should be divided in to three fire blocks (FB) of 300 m strip and 300 m interval like FB-1, FB - 2 and FB – 3. Outer boundary of plantation or grassland should not burnt in first year. Particular grassland will be divided into small strips then cutting and burning will be done under close supervision so that fire may not spread in unburnt patches. Same burning schedule should be followed for natural grassland.

6. *Eradication of tree species in natural grassland*

Natural succession of *khair, sissoo, sidha, siris* etc. in grassland of Jaldapara change the habitat type. To determine why cutting back or uprooting is necessary and what will be the future of grassland sample plots were laid out as after Pandit (1996, 2004). It is found from the analysis of data that total number of plants were 2992 per ha. and out of which 1579 were in height class 0.5m and less. 617 were in 0.5-2.00 m height class, 154 in 2.0m and above height class and 282 were in tree class (Table – 1).

To attain a tree size average time required is 5-8 years, irrespective of all species. So on an average after 5-8 years all natural grassland will be converted to wood land as a natural succession process.

7. *Maintenance of special habitat like dead trees (snags) and tall trees*

Special habitat within the sanctuary will be maintained as per following prescriptions.

i) Retention of 3-5 numbers hollow, dead top, partially dead or fully dead (snag) standing trees per ha. particularly trees >20 cm gbh and >5m tall. Such trees are used by a variety of wildlife species, particularly woodpeckers, barbets, nuthatches, hornbills and mammals.

ii) Retention of down wood size >20cm diameter at big end & >2 m long approximately 2-3 numbers per hectare.

iii) Retention of fruit and seed bearing trees and bamboo clumps.

iv) Retention of large well dispersed old over storey trees, approximately 2-5 trees per ha. Trees with deeply twisted, furrowed bark with peeling bark; many natural cavities should be retained.

v) Protection of riparian areas habitat along watercourses should be excluded from over wood removal or used as location of roads or other activities disruptive the vegetation.

vi) Caves, burrows and breeding sites should be maintained.

8. *Control of domestic livestock grazing of fringe villages and tea gardens inside the sanctuary*

Following strategies are important to control the illegal grazing.

i) Extensive patrolling.

ii) Raising of fodder plantations in community land, private land, vested land or Panchayat land for stall-feeding of cattle.

iii) Reduction in number of low yielding milch cattle through castration of useless bulls and artificial insemination of cows.

iv) Keeping of good health and disease free condition of village cattle through organization of veterinary camps and training to EDC members with the help of Animal Husbandry department or Animal Resource Development Department as Ecodevelopment activity.

v) Social fencing through fringe peoples cooperation.

vi) Development of cattle pawns.

vii) Awareness generation programme.

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DIVERSITY OF TANNASE PRODUCING BACTERIA IN THE FOREST SOIL OF *SHOREA ROBUSTA* Gaertn.f., PASCHIM MEDINIPUR, W.B., INDIA

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Abstract ■ The soil samples from various *Shorea robusta* Gaertn.f. forests of Paschim Medinipur District of West Bengal, India were studied for the isolation of total cultivable as well as tannase producing bacteria. Among the isolates, 42.85 % showed detectable tannase activity on selective tannic acid agar medium and among them percentages of good, moderate, and poor tannase producer were 3.33%, 5.71%, and 33.81 % respectively. The ratio of good tannase producer with that of total cultivable bacterial population is 1:31. On further screening, seven morphologically different and effective isolates were tested for tannase production through submerged fermentation (SmF). Tannase production was varied from 0.33 U/ml to 0.83 U/ml in SmF by the selected isolates. The highest tannase producer was identified as *Bacillus* sp.

Key words: Soil bacteria, Submerged fermentation, Tannase production

Introduction

Tannase (tannin-acyl-hydrolase, E.C. 3.1.1.20), an industrially important enzyme, is mainly used in the stabilization of malt polyphenols, clarification of beer, prevention of phenol induced maderization in wine and fruit juices (Bajpai and Patil 1997), manufacture of instant tea and reduction of antinutritional effect of tannins in animal feed. Tannase also gained paramount importance in biodegradation of tannin in natural environments like tannary effluents and wastewaters (Lekha and Lonsane 1997). It catalyzes the hydrolysis of ester and depside bonds of tannic acid into glucose and gallic acid (Das Mohapatra et al. 2005). Gallic acid has also several applications in chemical as well as pharmaceutical industries for the production of propyl gallate, pyrogallol, trimethoprim etc. (Das Mohapatra et al. 2006) but still it is an imported item of our country.

Though a number of tannase producing microorganisms were reported earlier (Lekha and Lonsane 1997, Bhat et al. 1998, Mondal and Pati 2000, Das Mohapatra et al. 2009) the search is still going on for obtaining the best. Reports on tannase production by fungi are more fre-

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quent in the literature than that of bacteria (Mondal et al. 2001a). In this regard Mondal et al. (2001a) mentioned that the tannase production from bacteria is more advantageous as they produce more enzyme within a short period of cultivation. In this present communication diversity of tannase producing bacteria of various *Shorea robusta* (Gaertn.f.) forests soil were studied and the best producer was identified.

Methods

Culture medium and conditions:

Tannase producing bacteria were isolated by plate dilution technique in tannic acid agar plate. The plate was prepared by selective tannic acid medium (STM), containing (g% w/v): Tannic acid, 1; NH_4Cl, 0.3; KH_2PO_4, 0.05 and $MgSO_4$, 0.05). and agar 3.0 %. The initial medium pH was adjusted to 5.0 after sterilization at 110°C for 30 min. The enzyme production capabilities of the seven isolates were studied separately through submerged fermentation. In each case organisms were grown in STM at 35°C for 24 h and next to that the broth was centrifuged (5000g for 5 min) and the pellet was washed twice in sterilized distilled water and used as inoculum for fermentation.

For submerged fermentation, 1% (v/v) inoculums was transferred to fresh STM and fermentation was carried out in 250 ml Erlenmeyer flask containing 50 ml liquid medium and incubated at 35°C in a rotary shaker (160 rpm) for 24 h. The culture supernatant obtained by centrifugation was assayed for enzyme activity. The growth of the organisms measured on the basis of biomass dry weight (mg/ml).

Assay of tannase:

Tannase activity in the fermented broth was determined by the colorimetric method of Mondal et al. (2001b). One unit of tannase activity was defined as the amount of enzyme, which is able to hydrolyze 1 µmol of ester linkage of tannic acid in 1 min at assay condition. All the experiments were performed in triplicate and the results represented here are the mean of the three. The standard deviations were within 10%.

Results and Discussion

Primarily two hundred ten colonies were randomly selected from different soil samples, from them ninety isolates were screened on the basis of significant clear zone formation around the colonies. Tannic acid agar plates are generally milky in appearance. Formation of clear zone around the colonies was due to hydrolysis of tannic acid by the tannase produced from the bacteria (Bradoo et al., 1996). A comparative record of tannase production by the isolates has been shown in Table – 1. Among the isolates 42.85 % showed detectable tannase activity and out of them 3.33%, 5.71%, and 33.81% were identified as good, moderate and poor tannase producer respectively. The ratio of good tannase producer with that of total bacterial population is 1:31. Morphological characteristics of the best

Table 1: Isolation and selection of tannase producing bacteria from the soil samples of *Shorea robusta* forest in tannic acid agar plate after 3-days of incubation at 35°C.

Diameter of tannic acid hydrolyzing zone (mm)	Category	Number of colonies	percentage
6 - 8	Good	7	3.33
3 – 5.5	Moderate	12	5.71
1 – 2.5	Poor	71	33.81
0	Nil	120	57.1

seven tannase producer are summarized in Table – 2. The characters studied showed that all are gram positive and nonmotile except PBK 16 and they produced white colour round colonies with entire margin. The strain PBK 16, 19, 29 were spore formers.

The growth and tannase producing ability of seven good selected isolates were presented in Fig. 1. Of these PBK 16 was found to be highest tannase producer and produced 0.83 U/ml enzyme in unoptimized state, which was 2.52 times more than the lowest (0.33 U/ml) producing strain, PBK 29. It was also found that enzyme production was directly correlated with the growth of the organism. Our finding is likely to be consistent with the observation of Vermeir and Vandamme (1988). The strain PBK 16 was identified in our laboratory following Bergey's Mannual of Systematic Bacteriology (1986) as *Bacillus* sp.

Generally microbes are sensitive to tannic acid, but we have been able to isolate several tannic acid resistant bacteria from different soil samples of *S. robusta* forest, which can degrade tannic acid and utilize it as an energy source. Tannase producers are usually predominant in soil samples of *S. robusta* forest (Mondal et al. 2001a) as *S. robusta* seed containing huge amount of tannin. Occurrence of tannase in soil microbes plays an active role in the decomposition and recycling of plant materials containing tannins.

Table 2: Morphological characteristics of the strains PBK – 12, 16, 19, 29, 64, 111 and 131.

Tests		Result of the strains						
		PBK 12	PBK 16	PBK 19	PBK 29	PBK 64	PBK 111	PBK 131
Colony Morphology	Configuration	Round	Round	Round	Round	Round	Round	Round
	Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire
	Elevation	Concave	Convex	Convex	Convex	Concave	Convex	Concave
	Surface	Smooth	Rough	Rough	Rough	Smooth	Rough	Smooth
	Density	TL	TL	TP	TP	TP	TL	TL
	Pigment	- Ve	- Ve	- Ve	- Ve	- Ve	- Ve	- Ve
Gram's Reaction		+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
	Shape	Circular	Rod	Rod	Rod	Circular	Rod	Rod
	Size	Short	Medium	Short	Short	Short	Long	Long
	Arrangement	Chain	Single	Single	Chain	Chain	Single	Chain
Spore	Endospore	-	+	+	+	-	-	-
	Position	-	Central	T	T & ST	-	-	-
	Shape	-	Oval	Oval	Oval	-	-	-
Motility		-	+	-	-	-	-	-
Fluorescence		-	-	-	-	-	-	-

T = Terminal, ST = Sub terminal, TL = Translucent, TP = Transparent

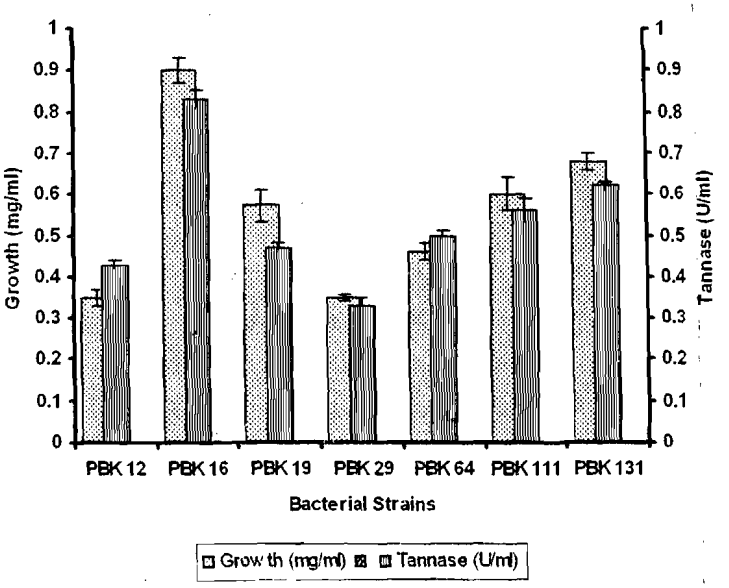

Fig. 1: Tannase production in submerged fermentation by seven bacterial strains at 35°C under shaking condition (160 rpm) for 24 h.

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