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Original Article

Biodiversity and Conservation Status of Fishes around Kalna and Its Adjacent Areas, Burdwan District, West Bengal, India

Indranil Bhattacharjee*, Sayantan Mukherjee*, Souvik Chatterjee, Papan Basak**, Surajit Ghosh**, Sujit Kar****

Abstract

A survey was conducted on biodiversity of fish fauna and their conservation status around Kalna and its adjacent area. Burdwan district, West Bengal, India with an objective to prepare a register of fresh water fishes and assess their conservation status. Regular bi-monthly sampling was conducted from January, 2015 to December, 2016 by the help of fishermen using indigenous fishing methods and by using different types of nets. Fishes were also collected from local fish markets. We have collected 28 fish species belonging to 7 orders, 13 families and 24 genera. Order Cypriniformes was the dominant group with 11 species followed by Perciformes 7 species, Siluriformes with 5 species, Osteoglossiformes with 2 species and Clupeiformes, Beloniformes and Synbranchiformes each with 1 species. Out of 28 species, 20 species are least concern, 4 species are near threatened, 2 are vulnerable and 1 each are under not evaluated and data is deficient category as per IUCN (2013) Red List category. According to CAMP (1998) conservation status, 13 species are at lower risk near threatened, 5 species are under not evaluated, 4 species are vulnerable, 3 at lower risk least concern, 2 species are endangered and 1 species is under exotic category. The maximum species richness (26) was recorded at Dhatrigram and low species richness (22) was recorded at Kana. The highest Shannon value was recorded to be (3.107) at Samdruagarh. The low Shannon value was (2.831) at Kalna.

Keywords: Fish Diversity; CAMP; IUCN Status; Biodiversity Indices; Kalna; West Bengal.

Introduction

Fish forms highest species diversity among all vertebrates besides its economic importance. India is one of the mega biodiversity hot spots (North East Region and Western Ghat) contributing about 11.72% of global fish diversity mainly from the greater Himalayan range on the northern plains, long stretches Eastern and Western ghats. Biodiversity is also essential for stabilization of ecosystem, protection of overall environmental quality, for understanding intrinsic worth of all species on earth (Ehrlich and Wilson, 1991). Important work has been done on fish diversity during the last few decades (Day, 1958; Jayaram, 1981; Menon, 1992; Shaji, 1995; Arunachalum, 2000; Daniel, 2001; Sarkar and Banarjee, 2000; Bhat, 2002; Mishra et al. 2003; Bossuyt et al. 2004; Rajalakshmi and Sreelatha 2006; Saha and Patra 2013; Bera et al. 2014; Bhattacharjee et al. 2016).

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Aquatic environments are experiencing serious threats to both diversity and ecosystem stability. Research is being persuade globally to develop systematic conservation planning to protect freshwater biodiversity (Margules and Pressey, 2000; Saunders et al., 2002, Nel et al., 2008). Various methods, strategies and priorities have been proposed (Cowx 1998; Lakra et al., 2006; Sarkar et al., 2008). Kottelat and Whitten (1996) considered the biological

change that environmental degradation brings about, and enumerated pollution, increased sedimentation, flow alteration, water diversion and introduced species as the main causes for decreased ichthyofaunal diversity in Asian countries.

Based on IUCN categories, the CAMP workshop (Molur and Walker, 1998) for fresh water fishes identified certain fish species which have attained the threatened/endangered status.

To achieve sustainable utilization of these resources, appropriate planning for biodiversity conservation and management strategies are of utmost importance and the greatest challenge. Therefore, scientific information about species, conservation status and ecosystems is essential for moving towards more sustainable use and scientific conservation efforts of our invaluable biological resources.

Materials and Methods

Study Area

Samplings are done from three sites around Burdwan. They are Site I: Samudragarh (23°34′N and 88°32′E); Site II: Dhatrigram (23°27′N and 88°31′E) and Site III: Kalna (23°22′N and 88°34′E).

Collection and Identification of Fishes

Sampling involved collection from various sites with the help of fishermen using indigenous fishing methods and by using different types of nets namely gill nets, cast nets and dragnets. Fishes were also purchased from the fishermen on the spot. We also visited local fish markets and look for the presence of any species which were not available during our experimental fishing. Immediately photographs were taken prior to preservation since formalin decolorizes the fish colour on long preservation. The specimens were preserved in 10% formalin and brought to the laboratory. They were fixed in formalin solution based on their size in separate jars. Smaller ones are placed directly while the larger ones were preserved after giving an incision on the abdomen before they were fixed in the formalin solution. Fishes were identified by using standard taxonomic keys for fishes of the Indian subcontinent (Day, 1958; Talwar and Jhingran, 1991; Jayaram, 1981, 1999). The fishes were labeled giving serial number, date of collection, place of collection, systematic position and common name on each jar. Conservation status of each fish was given based on the report on Conservation Assessment and Management Plan (CAMP) for freshwater fishes of India by Molur and Walker, 1998 and IUCN, 2013 Red List of Threatened Species.

Biodiversity Indices

Margalef richness index (M), Simpson's index (D), Simpson's Index of Diversity (1-D), Simpson's Reciprocal Index (1/D), Shannon's diversity index (H) and Pielou's evenness index (J), biodiversity indices were calculated (Chandra et al., 2015; Bhattacharjee et al. 2016).

Results

The periodical survey of the ichthyofauna revealed the occurrence of 28 fish species belonging to 7 orders, 13 families and 24 genera 3 were recorded over a period of one year, from January, 2015 to December, 2016 (Table 1). Among the collected species Order Cypriniformes was the dominant group with 11 species followed by Perciformes 7 species, Siluriformes with 5 species, Osteoglossiformes with 2 species and Clupeiformes, Beloniformes and Synbranchiformes each with 1 species. Number of fish species under different categories of threat as per CAMP / IUCN is presented in Figure 1. Out of 28 species, 20 species are least concern, 4 species are near threatened, 2 are vulnerable and 1 each are under not evaluated and data is deficient category as per IUCN (2013) Red List category. Percentage of fish species under different categories of threat as per IUCN Status is presented in Figure 2. According to CAMP (1998) conservation status, 13 species are at lower risk near threatened, 5 species are under not evaluated, 4 species are vulnerable, 3 at lower risk least concern, 2 species are endangered and 1 species is under exotic category. Percentage of fish species under different categories of threat as per CAMP Status is presented in Figure 3. The data of Diversity Indices are presented in Table 2. The maximum species richness (26) was recorded at Dhatrigram and low species richness (22) was recorded at Kana. The highest Shannon value was recorded to be (3.107) at Samdruagarh. The low Shannon value was (2.831) at Kalna. Habitat loss and environmental degradation has seriously affected the fish fauna. Recent data regarding fish diversity of the study site, aiming to contribute a better knowledge of the fish diversity and a tool for conservation planning of aquatic environments in this region. To maintain fish biodiversity has an immense importance as it is not always possible to identify individual species critically to sustain aquatic ecosystem.

Table 1: Fish species collected at three different sites with their names (common and scientific) and conservation (CAMP and IUCN) Status

Common Name	Scientific Names	Samduragarh	Dhatrigram	Kalna	Order	Family	CAMP Status	IUCN Status
Rohu	*Labeo rohita* Hamilton	14	13	14	Cypriniformes	Cyprinidae	LRnt	LC
Catla	*Catla catla* Hamilton	12	13	11	Cypriniformes	Cyprinidae	VU	LC
Mrigal carp	*Cirrhinus mrigala* Hamilton	11	11	12	Cypriniformes	Cyprinidae	LRnt	VU
Kalbose	*Labeo calbasu* Hamilton	13	10	3	Cypriniformes	Cyprinidae	LRnt	LC
Bata	*Labeo bata* Hamilton	13	6	11	Cypriniformes	Cyprinidae	LRnt	LC
Silver carp	*Hypophthalmichthys molitrix* Valenciennes	13	4	0	Cypriniformes	Cyprinidae	E	NT
Spotfin swamp barb	*Puntius sophore* Hamilton	11	12	12	Cypriniformes	Cyprinidae	LRnt	LC
Mourala	*Amblypharyngodon mola* Hamilton	12	4	4	Cypriniformes	Cyprinidae	LRlc	LC
Common carp	*Cyprinus carpio* Linnaeus	12	11	2	Cypriniformes	Cyprinidae	NE	VU
Kalbose	*Labeo calbasu* Hamilton	0	5	10	Cypriniformes	Cyprinidae	LRnt	LC
Two spot barb	*Puntius ticto* Hamilton	0	6	0	Cypriniformes	Cyprinidae	LRnt	LC
Flat head goby	*Glossogobius giuris* Hamilton	13	2	2	Perciformes	Gobidae	LRnt	LC
Climbing Perch	*Anabas testudineus* Bloch	12	8	11	Perciformes	Anabantidae	VU	DT
Dwarf gourami	*Colisa lalia* Hamilton	12	12	5	Perciformes	Osphronemidae	NE	LC
Glassy perchlet	*Chanda nama* Hamilton	12	3	6	Perciformes	Ambassidae	NE	LC
Snakehead Murrel	*Ophiocephalus striatus* Bloch	12	12	10	Perciformes	Channidae	LRlc	LC
Spotted snake head fish	*Ophiocephalus punctata Bloch*	14	2	0	Perciformes	Channidae	LRnt	LC
Nile tilapia	*Oreochromis niloticus* Linnaeus	13	14	11	Perciformes	Chichlidae	NE	NE
Tangra	*Mystus tengara* Hamilton	11	8	6	Siluriformes	Bagridae	NE	LC
Freshwater cat fish	*Wallago attu* Bloch & Schneider	12	2	2	Siluriformes	Siluridae	LRnt	NT
Pabo cat fish	*Ompok pabo* Hamilton	0	0	2	Siluriformes	Siluridae	EN	NT
Stinging cat fish	*Heteropneustes fossilis* Bloch	12	3	2	Siluriformes	Siluridae	VU	LC
Walking cat fish	*Clarias batrachus* Linnaeus	13	2	3	Siluriformes	Clariidae	VU	LC
Bronze featherback	*Notopterus notopterus* Pallas	11	2	1	Osteoglossiformes	Notopteridae	LRnt	LC
Indian featherback	*Chitala chitala* Hamilton	0	2	0	Osteoglossiformes	Notopteridae	EN	NT
Indian river shad	*Gudusia chapra* Hamilton	0	2	0	Clupeiformes	Clupeidae	LRlc	LC
Freshwater gar fish	*Xenentodon cancila* Hamilton	12	0	0	Beloniformes	Belonidae	LRnt	LC
Indian spiny eel	*Macrognathus pancalus* Hamilton	12	3	2	Synbranchiformes	Mastacambelidae	LRnt	LC

CAMP Status: Lower Risk near threatened (LRnt); Vulerable (VU); Lower Risk least cocern (LRlc); Exotic (E); Not Evaluated (NE); Endangered (EN)
IUCN Status: Least Concern (LC); Vulnerable (VU); Near Threatened (NT); Data deficient (DT); Not Evaluated (NE)

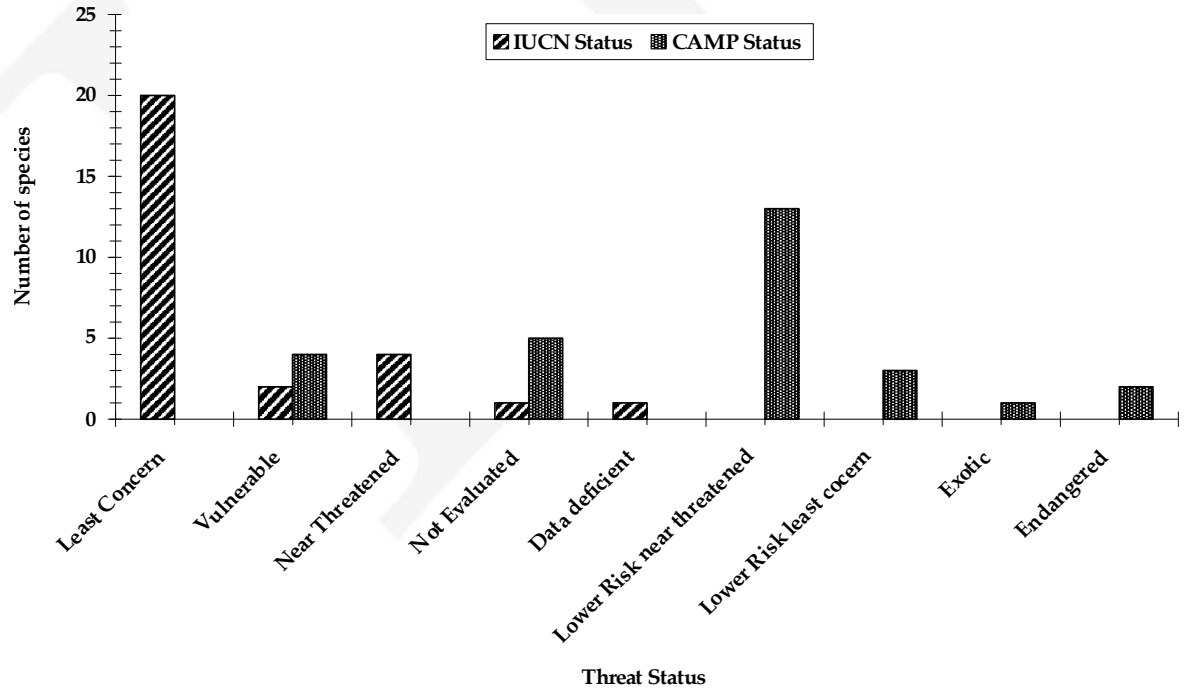

Fig. 1: Number of fish species under different categories of threat as per CAMP / IUCN

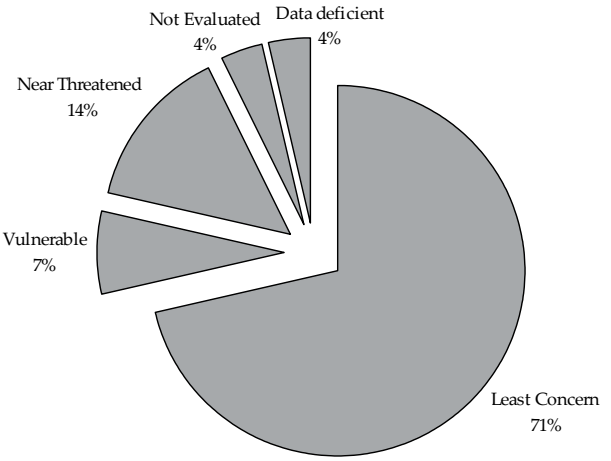

Fig. 2: Percentage of fish species under different categories of threat as per IUCN Status

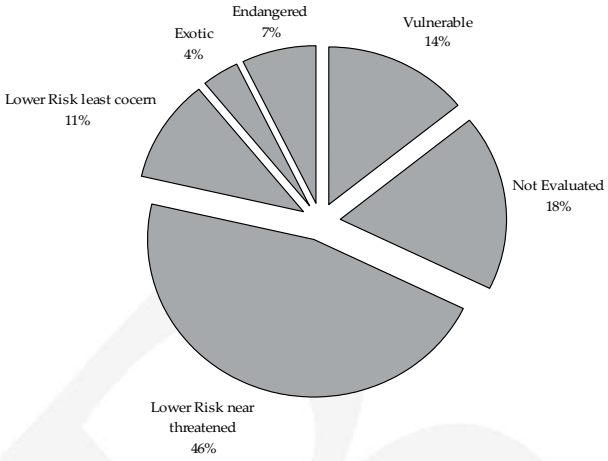

Fig. 3: Percentage of fish species under different categories of threat as per CAMP Status

Table 2: Biodiversity Indices of fish species at three different sites

Indices	Samduragarh	Dhatrigram	Kalna
Total No. of Species (S)	23	26	22
Total No. of Individuals (N)	282	172	142
Natural Log of Species (ln S)	3.13	3.25	3.09
Natural Log of Individuals (ln N)	5.64	5.14	4.95
Margalef's Index (M)	3.90	4.86	4.24
Simpson's Index (D)	0.040	0.046	0.059
Simpson's Index of Diversity (1-D)	0.960	0.954	0.941
Simpson's Reciprocal Index (1/D)	25	21.73	16.94
Shannon Index (H)	3.107	2.982	2.831
Pielou's Index (J)	0.992	0.917	0.916

Discussions

Damming, deforestation, diversion and withdrawal of water for irrigation, urban and industrial consumption have caused large scale changes in the channel bed and hydrology of the river in terms of flow, flow-rate, flood-rhythm and regime. The upland fast-moving habitat has been lost to reservoirs which are unfavorable for rheophilic species (Sarkar et al., 2008). Reckless killing by stupefying methods of brood fishes in spawning season and juveniles during post-monsoon periods have affected a number of food and game fishes. Over-fishing affects heritable life history parameters like growth and age of sexual maturity. Over-exploitation of fishery resources due to its higher economic value has increased the vulnerability of the population in different ecosystems. Global climate change is likely to result in severe droughts and floods with major impact on human health and food supplies, according to the India's report to the United Nations (Xenopoulos, 2005) reduction in river discharge due to combined effect of climate change and water withdrawal will make up to 75% global freshwater fish biodiversity to become extinct by 2070.

Conclusions

Fish biodiversity conservation represents major environmental challenge at the global level, and will continue under threat if there is no strenuous policy action to curb human activity. Important management plans have been considered from the study for the conservation of fish biodiversity in the freshwater body which should be inserted into the fishery policies of the Government, such as, identification and listing of threatened and endangered fish species of freshwater body, determination of population size and distribution, find out the breeding behavior of threatened fish species which is essential for both *ex situ* and *in situ* conservation of the species, development of techniques of captive breeding and brood stock maintenance of fishes of potential economic importance.

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Original Article

Evaluating Morphological, Physiological and Biochemical Responses of Three Amphiploid Brassica Species to Salinity Stress

Abhijeet Saxena*, Ram Singh Purty**

Abstract

Brassica occupy third place among the various oilseed species but their productivity, growth and oil production are greatly reduced due to salinity. Amphidiploid Brassica species includes *B. napus* (AC genome), *B. juncea* (AB genome) and *B. carinata* (BC genome) are more tolerant to salinity as they are derived from diploid species which include *B. campestris* (AA, n=10), *B. nigra* (BB, n=8) and *B. oleracea* (CC, n=9). Screening of available local cultivars may facilitate us in identification of varieties suitable for that particular area. Therefore, in the present study different parameters such as growth, electrolyte leakage, K^+/Na^+ ratio, chlorophyll, protein, malondialdehyde and proline content were used to study the effects of 200 mM NaCl for 24 h on the seedlings of available local three amphiploid *Brassica* species i.e., *B. juncea* L. cv Pusa Bold, *B. carinata* cv Pusa Gaurav (DLSC 1) and *B. napus* var Neelam (HPN-3). Correlation amongst the different parameters tested for screening salinity tolerant was also studied. In the present investigation, growth, chlorophyll and protein content of the seedlings decreased sharply in all the species upon salinity treatment. Electrolyte leakage analysis indicated that membrane damage of *B. juncea* seedlings was least whereas endogenous K^+/Na^+ ratio was found to be higher. Strong positive correlation between the electrolyte leakage and malondialdehyde content analysis has been obtained ($r=0.9$). The response of all the three amphiploid *Brassica* species under salinity condition differed significantly ($p \leq 0.01$). Amongst the three amphiploid species *B. juncea* L. cv Pusa Bold was found to be more tolerant.

Keywords: Abiotic Stress; Brassica; Electrolyte Leakage; Lipid Peroxidation; Proline.

Introduction

Plants exposed to the natural environment generally encounter various abiotic stresses which includes low and high temperature, drought, salinity, or the biotic stress like viruses, insects, nematodes, bacteria, fungi etc. These stresses greatly affect the plant productivity and it has been estimated that almost 50% of the crop yield is reduced due to abiotic stress whereas around 20-30% by biotic stress [1]. Amongst the various abiotic stresses soil salinity greatly affect the crop productivity [2].

In the past several efforts have been made for the development abiotic stress tolerance crop using transgenic technology but only little success has been achieved owing to its multigenic and quantitative nature [3]. Salinity tolerance is a complex process, it involves several physiological, molecular and biochemical process, and the level varies from species or amongst varieties within same species [4-8].

Under salinity stress, the morphological changes are the first response which can be seen very clearly [9]. However, these changes may not be enough to differentiate amongst species or varieties within same species. It is important to investigate the physiological, biochemical or molecular changes such as relative water content, toxic ions, osmotic potential,

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photosynthetic pigment, activity of enzymes, proteins/genes expression under salinity condition in order to understand the mechanism of salinity tolerance in plants [10-13].

One of the methods for understanding the mechanism and developing salinity tolerance in crop plants is by screening of available exotic cultivars of crop plants for salinity tolerance [14-17]. It has two major advantages, ûrst the tolerant genotype thus made available can be used in breeding programs and second, a comparative analysis at physiological/biochemical and/or molecular level of these contrasting cultivars can help us in understanding and unraveling novel survival mechanisms [18,19].

Brassica occupy third place among the various oilseed species but their growth, yield, and oil production are markedly reduced due to salinity. There is significant inter and intraspecific variation for salt tolerance within *Brassica* species which includes both amphidiploids and diploid species [20, 21]. An amphidiploid species *Brassica napus* (AC genome, n = 19) is derived from hybridization between *B. rapa* (A genome, n = 10) and *B. oleracea* (C genome, n = 9), *B. juncea* derived from *B. rapa* (A genome, n = 10) and *B. nigra* (B genome, n = 8) and *B. carinata* derived from *B. oleracea* (C genome, n = 9) and *B. rapa* (A genome, n = 10) [22].

Since the amphidiploid species were derived from diploid species it can be expected that the amphidiploid will have traits from both the parents and tolerate salinity much better than diploid species [23]. Therefore, the present investigation was carried out in order to determine variations in degree of salt tolerance amongst amphidiploid *Brassica* at the seedling stages.

Materials and Methods

Plant Material and Germination

Seeds of three different amphidiploids *Brassica* species i.e., *B. juncea* L. cv Pusa Bold, *B. carinata* cv Pusa Gaurav (DLSC 1) and *B. napus* var Neelam (HPN-3) were procured from Indian Agricultural Research Institute (IARI), New Delhi. Seeds were washed with de-ionized water and surface sterilized with 0.1% $HgCl_2$ and Bevastin. Seeds were allowed to germinate in a hydroponic system for 48 h in dark and then transferred to light for further growth under control conditions (25 ± 2^0C, 12 h light and dark cycles) in plant growth chamber.

Salinity Stress Treatment

For salinity stress treatment, 7 days old seedlings were treated with 200 mM NaCl for 24 h using hydroponic system. Simultaneously, seedlings maintained in de-ionized water were taken as control. After stress treatment, seedlings were harvested for growth analysis, electrolyte leakage analysis, Na^+ and K^+ estimation, chlorophyll assay, MDA assay, protein content analysis and proline assay.

Growth Analysis

To study the effect of salinity stress on seedling growth after 24 h of salt treatment, the root and shoot length of the seedlings were compared with unstressed control seedlings. Since the various species analyzed in this study had different rates of growth under control conditions, comparison of these species was based on the relative percentage change which was calculated by applying the formula [(Control – stressed)/ Control] x 100.

Electrolyte Leakage

Membrane damage due to salinity stress was evaluated by measuring electrolyte leakage as previously described [24]. After 24 h, stressed as well as unstressed seedlings were harvested and washed with distilled water to remove surface ions. Around 100 mg tissue was dipped in 20 ml of distilled water and incubated at 32°C for 2h. The initial electrical conductivity (E1) of the immersion solution was measured using conductivity meter (Ri Digital Conductivity Meter, Model 215-R). Then the seedlings along with immersion solution were autoclaved for 15 min at 121°C, cooled, and final electrical conductivity (E2) was measured. The relative electrical conductivity was calculated by the formula (E1/E2) x100.

Estimation of Na^+ and K^+ Contents

Determination of endogenous Na^+ and K^+ contents was done using Flame photometer following the protocol previously described [24]. Around 100 mg of seedling tissue (unstressed or salinity stressed) of each of the three *Brassica* species were predigested by soaking overnight with 10 ml of concentrated HNO_3 and finally digested with di-acid mixture (20 ml) containing HNO_3 and $HClO_4$ acid (9:4). The digested material was cooled, diluted with distilled water and filtered through Whatman No. 42 filter paper. The volume of the ûltered extract was made upto 30 ml with distilled water and was used to measure specificions.

Chlorophyll Estimation

Unstressed and stressed seedlings were harvested and approximately 50 mg leaves were used for the extraction. Chlorophyll pigments were extracted by immersing leaves in tube containing 1 ml of 80% acetone for 12 h in the dark at 4^0C. Supernatant were obtained after centrifugation at 5000 g for 5 min at room temperature and absorbance was recorded at 663 nm and 645 nm using spectrophotometer (Spectra Max M2). The amount of chlorophyll a and chlorophyll b was calculated according to the protocol described by Arnon [25]. The relative percentage decline in chlorophyll a and chlorophyll b was calculated by applying the formula [(Control – stressed)/ Control] x 100.

Total Protein Content Analysis

Salinity stressed and unstressed seedlings were harvested in liquid nitrogen and grinded to powder in mortar and pestle. Protein was extracted using phosphate extraction buffer (0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, d.H_2O, pH 7.2). Supernatant obtained after centrifugation at 9000 g at 4^0C for 15 minutes was used for estimation by Bradford assay [26]. Concentration of total protein in the extract was estimated by measuring the absorbance at OD_{595nm} using spectrophotometer (Spectra Max M2).

Malondialdehyde Estimation using TBARS Assay

Malondialdehyde **(**MDA) was extracted from approximately 50 mg seedlings (unstressed and stressed) using 1 ml 0.25% Thiobarbituric acid (TBA) dissolved in 10% trichloroacetic acid [27]. Tube containing extract was incubated at 85°C for 30 min and then immediately chilled on ice. Supernatant was obtained after centrifugation at 10,000 g for 10 min and absorbance was recorded at 532 nm (MDA-TBA complex) and 600 nm spectrophotometer (Spectra Max M2). The OD_{600} values were subtracted from MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law with an extinction coefficient εM =155/mM/cm. The level of lipid peroxidation is expressed as μM MDA/g.

Proline Estimation

Proline was extracted from both stressed and unstressed seedlings following the protocol previously described [28]. Around 100 mg seedlings were homogenized in 5 ml of 3% (w/v) aqueous sulphosalicylic acid and centrifuged at 12 000 g for 10 min to obtain supernatant. The reaction mixture (1 ml supernatant: 1 ml acid-ninhydrin : 1 ml glacial acetic acid) was incubated at 100°C for 1 h and terminated immediately by transferring to ice bath. The reaction mixture was extracted with 2 ml Toluene, mixed vigorously and allowed to cool down at room temperature for 30 min until separation of the two phases. The optical density of an upper phase was measured at 520 nm using toluene for a blank. The proline content was determined from a standard curve using pure proline.

Statistical Analysis

All experiments were repeated three times. For each experiment at least 10 seedlings were taken. Results are presented as mean ± S.E. Analysis of variance of data and their correlation between different parameters were calculated using the built-in data Analysis ToolPak in MS Excel.

Results

Effect of Salinity Stress on Seedling Growth

Upon 200 mM NaCl treatment, seedlings of *B. juncea* L. cv Pusa Bold, *B. carinata* cv Pusa Gaurav (DLSC 1) and *B. napus* var Neelam (HPN-3) responded differently (Figure 1). Under the imposed of 200 mM NaCl stress treatment for 24 h seedlings of all the cultivars lost their turgidity and color of leaves turned yellow and tip turned pale. All the species differed significantly in growth in response to 200mM NaCl for 24 h ($p \leq 0.01$). The growth of all the *Brassica* species reduced when compared to unstressed control (Figure 1). The seedlings showed reduction in both root and shoot length upon 24h of salinity stress. In order to study the effect of salinity stress on reduction in root and shoot length of seedlings the relative percentage reduction were calculated. The relative percentage reduction in root length was found to be 10.2%, 18.99% and 18.90% for *B. juncea B. carinata* and *B. napus,* respectively (Figure 2a). Similarly, the relative percentage reduction in shoot length was found to be 10.44%, 28.35% and 17.06% for *B. juncea B. carinata* and *B. napus,* respectively (Figure 2b). Stress treatment caused excessive wilting of seedlings of *B. carinata* and *B. napus* in compare to *B. juncea.*

Effect of NaCl on Membrane Stability

Stability of cell membrane significantly differed amongst cultivars, in response to 200mM NaCl for 24 h ($p \leq 0.01$). Electrolyte leakage in seedlings of *Brassica carinata* was found to be very high (82%) compared to

other two *Brassica* species (Figure 2c). *Brassica juncea* exhibited a minimum electrolyte leakage of 53% whereas it was 65% for *Brassica napus*. This analysis clearly indicates that cell-membrane stability was least affected under salinity stress in seedlings of *Brassica juncea.*

Effects of Salinity on Cytosolic Na^+ and K^+ Content

In the present investigation endogenous Na^+ and K^+ content was estimated in salinity stressed seedlings. Endogenous Na^+ and K^+ content differed significantly amongst *Brassica* species ($p \leq 0.01$). Accumulation of Na^+ content was found to be 1.8, 4.2 and 3.5 mg g^{-1} FW for *B. juncea B. carinata* and *B. napus,* respectively. All the *Brassica* species maintained high K^+ content which was found 8.4, 7.6 and 7.4 mg g^{-1} FW for *B. juncea B. carinata* and *B. napus,* respectively. It was observed that *Brassica juncea* maintained high K^+/Na^+ ratio as compared to other two *Brassica* species (Figure 2d).

Effects of Salinity on Chlorophyll Content

Salinity induced decline in chlorophyll content was observed in all the *Brassica* species. Under salinity stress treatment chlorophyll a content was found to be 0.38, 0.24 and 0.14 mg g^{-1} DW compared to the unstressed control value of 0.47, 0.35 and 0.38 mg g^{-1} DW resulting in chlorophyll a reduction of 19.14%, 31.42% and 63.15% in *B. juncea*, *B. napus* and *B. carinata,* respectively (Figure 2e). Similarly, percentage decline in chlorophyll b content was also calculated. Under salinity stress, chlorophyll b content was found to be 0.19, 0.12 and 0.09 mg g^{-1} DW compared to the unstressed control value of 0.3, 0.18 and 0.21 mg g^{-1} DW resulting in chlorophyll b reduction of 36.66%, 33.33% and 57.14% in *B. juncea*, *B. napus* and *B. carinata,* respectively. Thus, the percentage decline in chlorophyll a and chlorophyll b content was maximum in *B. carinata* and least in *B. juncea.*

Effects of Salinity on Total Protein Content

Under salinity stress treatment protein content decreased in all the *Brassica* species. It was noted that under salinity stress *B. juncea* maintained higher protein content than *B. carinata* and *B. napus*. Protein content under salinity stress decreased significantly by 13.62%, 22.60% in *B. juncea*, *B. napus* and maximum of 57.21% in *B. carinata,* respectively (Figure 2f).

Effects of Salinity on Lipid Peroxidation

The lipid peroxidation was measured by determining the level of malondialdehyde (MDA) as indicator of lipid peroxidation. MDA content was increased under salinity stress treatment for 24 h in all the *Brassica* species. Percentage increase in MDA content was 20%, 31.69% and 62.4% for *B. juncea*, *B. napus* and *B. carinata,* respectively (Figure 2g).

Effects of Salinity on Proline Content

Under salinity stress treatment for 24 h, accumulation of proline amongst *Brassica* species differed significantly ($p \leq 0.01$). Proline content accumulation was found to be 0.66, 0.43 and 0.21 mg g^{-1} DW in *B. juncea*, *B. napus* and *B. carinata,* respectively. Percentage increase in proline content was 86.3%, 81.7% and 71.8% for *B. juncea*, *B. napus* and *B. carinata,* respectively (Figure 2h).

Fig. 1: Effect of 200 mM NaCl for 24h in the Brassica seedlings. (1) *B. juncea* unstressed control, (2) *B. juncea* stressed, (3) *B. carinata* unstressed control, (4) *B. carinata* stressed, (5) *B. napus* unstressed control, (6) *B. napus* stressed.

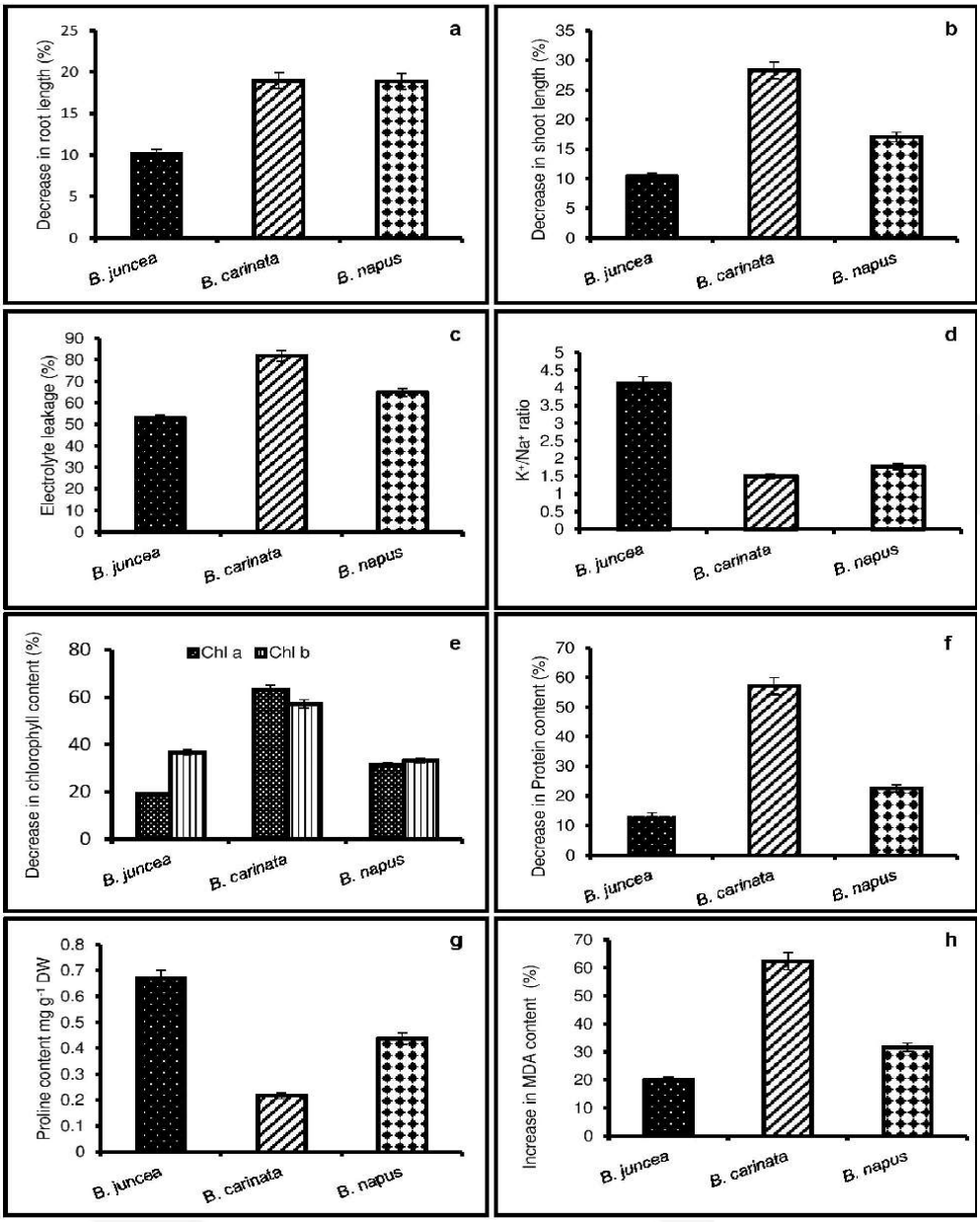

Fig. 2: Analysis of various morphological and biochemical parameters in the Brassica seedlings after 200mM NaCl treatment for 24 h. (a) Relative percentage decrease in root length. (b) Relative percentage decrease in shoot length. (c) Percentage change in electrolyte leakage. (d) Ratio of K^+/Na^+. (e) Relative percentage decrease in chlorophyll content. (f) Relative percentage decrease in protein content. (g) Endogenous proline content (mg g^{-1}DW). (h) Relative percentage increase in MDA content.

Discussion

Soil salinity is a major factor that reduces the productivity of crop worldwide [29]. Soil salinity is caused may be due to two reason; firstly, due to high rate of evapotranspiration and secondly, when leaching of the inorganic salts from the soil surface is very less resulting in the increase of soil salinity and sodicity [30]. The use of poor quality irrigation water is also the greatest cause of salinity [3]. Any change in soil condition can easily be sensed first of all by root organ system and the message is then conveyed to other parts of the plant like the tissues in shoot and leaves [31]. In the present study, decrease in root and shoot length were observed when seedlings were grown under salinity condition as observed in the decrease in plant length when treated with 200 mM NaCl. Reduction of plant growth by salinity differs between species and even between varieties and cultivars due to variability of salt tolerance among domestic and wild germplasms [7]. In the present

study, all the *Brassica* species responded differently upon salinity treatment. The relative percentage decrease in growth was found maximum for *B. carinata* cv Pusa Gaurav (DLSC 1) compare to other two amphidiploids species. Reduction in plant growth is due to reduction in the osmotic potential that restricts the absorption of water and nutrients by roots [32]. Salinity stress causes accumulation of salt ions in cells that causes toxicity and this can clearly visible in plants by chlorosis and necrosis of the leaf tissues [33]. In the present study, salinity stress not only brought about change in the growth but also changed the color of leaves to yellow and lost turgidity which was very clear among all the *Brassica* cultivar compared to unstressed control. These changes are due to decrease in chlorophyll content and water content of cells, resulting reduction in photosynthetic activity and turgidity [34]. Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important [35]. Turgidity lost due to salinity stress may cause injury in the cell membrane. The technique for the estimating the membrane damage is measuring the solute or ions leached out from the cell upon injury. Electrolyte leakage measurement is the indication of amount cell injury or membrane damage. Integrity or stability of cell membrane may vary amongst species or varieties of same species. In the present study all the *Brassica* species responded differently under salinity stress. *Brassica juncea* cv. Pusa Bold exhibited a minimum electrolyte leakage compared to other two *Brassica* species which clearly indicates that cell-membrane stability was least affected under salinity stress. A robust membrane can selectively restrict the entry of Na^+ ions into cells which is one of the key features of plant salt tolerance thereby maintaining the optimal K^+/Na^+ ratio in the cytosol [36]. Under salinity conditions, absorption of Na^+ and Cl^- competes with nutritional elements such as K^+, N, P, and Ca^{2+} by plants, resulting in ionic imbalance in the cell [37]. More Na^+ enters the cell due to similar in the hydrated ionic radii between Na^+ and K^+ makes it difficult for the transporter to discriminate between the two ions [38, 39]. However, tolerant plants overcome restricting the entry of Na^+ ions into cells, extrusion of Na^+ ions out of the cell or/and vacuolar compartmentation of Na^+ ions. In the present study, *B. juncea* cv. Pusa Bold maintained high ratio of K^+/Na^+ ratio compared to other two *Brassica* species possibly exhibiting combination of these strategies and is hence able to maintain favourable K^+/Na^+ ratio. Several proteins or transporter have been reported to play important role in pumping out excess of Na^+ ions out of the cell in tolerant plant.

Lipid peroxidation has been associated with cell damages caused by different biotic and abiotic stresses and is often used as an indicator of salt-induced oxidative damage to the cellular membranes [40]. Therefore in the present study, lipid peroxidation was measured by determining the level of malondialdehyde (MDA) content. There was a strong positive correlation between the electrolyte leakage and MDA content under salinity stress (r=0.9). The species showed maximum electrolyte leakage had higher MDA content. However, salinity stress may have positive or negative effect on protein content. In the present investigation, under salinity stress treatment protein content decreased in all the *Brassica* species. Excess of Na^+ content in the cell may degrade enzymes/proteins thereby affecting the whole biochemical and cellular process. Protein content was found to be higher in *B. juncea* compare to *B. carinata* cv Pusa Gaurav (DLSC 1) and *B.napus* var Neelam (HPN-3) indicating that *B. juncea* cv. Pusa Bold has better mechanism for overcome NaCl stress than other two species. Tolerant plant tries to maintain higher protein content because proteins serve as a reservoir of energy or may be adjuster of osmotic potential in plants subjected to salinity [41]. Under salinity stress, level of total free amino acids was reported to be higher in the leaves of salt tolerant in compare salt sensitive lines of sunflower, *Eruca sativa* and *Lens culinaris* [42-44]. Many amino acids including proline, alanine, arginine, glycine, serine, leucine, and valine and the non-protein amino acids and amides accumulate in plants exposed to salt stress [41]. Proline is a major amino acid that accumulates in the cytosol during salinity stress and accomplished osmotic adjustment [45-47].

Conclusion

Salinity tolerance is a complex process it is a cumulative effect of responses at physiological, molecular and biochemical levels. Therefore, in order to understand the molecular mechanism of salinity tolerance in plants it is important to investigate different parameters like RWC, ions contents, photosynthetic pigment, cell membrane injury, activity of enzymes, proteins/genes expression under salinity condition. In the present investigation, *B. juncea* L. cv Pusa Bold tolerated salinity better than other two amphidiploids *Brassica* species i.e., *B. carinata* cv Pusa Gaurav (DLSC 1) and *B. napus* var Neelam (HPN-3). Screening of available local/exotic cultivars and comparing them at physiological/ biochemical and/or molecular level will help us in understanding and unraveling novel survival mechanisms.

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Original Article

Effect of Variable Concentration of Dissolved Oxygen (DO) on Population Growth Rate and Food Conversion Ratio in Fish: Model "Molly" [*Poecilia sphenops* (Valenciennes, 1846)]

Santi Ranjan Dey

Abstract

Commercial aquaculture is growing worldwide. With fisheries reaching a stagnating phase, India will have to look to aquaculture in different way, in the future to provide fish products that will likely be needed. In view of this, a study on water quality management was done which specifically looked at the effects of dissolved oxygen (DO) on fish population growth and increase of body mass. The study was done by using Molly (*Poecilia sphenops*) in fresh water habitat. In the case study, 120 molly of 0.5 - 1 g. in weight were reared in replicate at 40%-100% DO levels in different aquarium of 10 liter capacity. Every alternate day 5 gm of food was given. The DO was regularly measured by Wrinkler's method. The subsequent effect of oxygen saturation levels on growth and feed conversion ratios were taken in three, six, nine and twelve months. The results showed that oxygen saturation level had an effect on the reproduction, growth and food conversion ratio. Below 60%, the population growth was comparatively lower. Feed conversion ratio was higher at 60% and above, compared to lower oxygen level. However it is found that at highest DO level the body mass increase is little lower than medium DO level (75.1%). The rate of population increase is highest at DO 88 % but the body mass increase is little lower (91%) in that DO. The conclusion is that oxygen saturation level has a positive correlation with population growth and food conversion to body mass ratios of fish. But at high DO condition the fish probably increase its activity and metabolism.

Keywords: Dissolve Oxygen; Fish; FCR; Molly; Physiology; Respiration.

Introduction

The contribution of aquaculture to global supplies of fish, crustaceans, molluscs and other aquatic animals is growing more rapidly than all other animal food-producing sectors (Balarin, 1985). This production has greatly outpaced population growth, with a per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.1 kg in 2004, representing an average annual population growth rate of 7.1% (FAO 2006a). With fisheries reaching a stagnating phase, India will have to look to aquaculture in different way, in the future to provide fish products that will likely be needed. The fisheries sector alone contributes nearly a third of the world's supply of fish products (FAO, 2006b). Unlike terrestrial farming, where the bulk of the production is based on a limited number of species, aquaculture is based upon near about 220 species. India is endowed with vast freshwater consisting 45,000 Km. of rivers, 26,334 Km. of canals, ponds and tanks 2.36 million hectares and 2.05 million hectares of reservoirs, which present like harbor a rich and diversified fish fauna characterized by many rare and endemic fish species. About 21,730 species of fishes have been recorded in the world; of which, about 11.7% are found in Indian waters. Out of the 2546 species so far listed, 73 (3.32%) belong to the cold freshwater regime, 544 (24.73%) to the warm freshwater domain, 143 (6.50%) to the brackish water and 1440 (65.45%) to the marine ecosystem. The freshwaters of India have been viewed from a single perspective: that of economic production (Timmons

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et al, 2014). They are to be sources of irrigation or urban-industrial water supply or of hydro power; they are to receive sewage and industrial waste; they may produce edible fish. In view of this, a study on water quality management was done which specifically looked at the effects of dissolved oxygen (DO) on fish population growth and increase of body mass (Randolph and Clemens. 1976). Water quality may be a major factor in the high mortality rates of fry and fingerlings and therefore hopefully the knowledge obtained here can be used to monitor the water quality parameters to possibly solve this problem (Svobodova *et al*, 1993, Tom, 1998). The main objective of the study was to gain knowledge of water quality management for a particular fish population in commercialized aquaculture.

The objective of the study are divided in various questions, does oxygen saturation have any effect on population growth rate of fish? If yes, at which levels is the growth affected positively? Whether, there is any effect of the oxygen saturation on the food conversion to body mass ratio in fish? At which saturation level is the food conversion ratio best?

Water Quality in Aquaculture

Water quality is the totality of physical, physiological, biological and chemical parameters that affect the growth and welfare of cultured organisms. Quality of water is, therefore, an essential factor to be considered. Although the environment of fish aquaculture is a complex system, consisting of several water quality variables, only few of them play decisive role. The critical parameters are temperature, suspended solids and concentrations of dissolved oxygen, ammonia, nitrite, carbon dioxide and alkalinity. However, dissolved oxygen is the most important and critical parameter, requiring continuous monitoring in aquaculture production systems. This is due to fact that fish aerobic metabolism requires dissolved oxygen (Ultsch *et al*, 1978).

Gas Exchange and Oxygen Concentration in Water

Oxygen as a gas has a low solubility in water. In addition, its amount contained in water varies with temperature and salinity in a predictable manner. Less oxygen can be held in fully air-saturated warm sea water than fully air-saturated cold freshwater, while the oxygen content of water sets the absolute availability of oxygen in the water. It is the oxygen partial pressure gradient that determines how rapidly it can move from water into the fish's blood to support its metabolic rate. This is because oxygen moves by diffusion across the gills of fish. According to Fick's law of diffusion, the rate of oxygen diffusion across the gills is determined by the gill area, diffusion distance across the gill epithelia, diffusion constant and difference in partial pressure of oxygen across the gills. Consequently, partial pressure of oxygen is the most appropriate term for expressing oxygen levels in water. However, oxygen concentration is more commonly used term and, for a given temperature and salinity, the partial pressure of oxygen and oxygen content in water are linearly related (Wedemeyer, 1996).

Oxygen Uptake in and Carbon Dioxide Release from the Fish

During respiration, fish take in oxygen and give out carbon dioxide. The process is done by using gills in almost all fish although some can also use some other parts of the body in addition to gills. When a fish respires, a pressurized gulp of water flows from the mouth into a gill chamber on each side of the head. Gills themselves, located in gill clefts within the gill chambers, consist of fleshy, sheet like filaments transected by extensions called lamellae. As water flows across the gills, the oxygen within them diffuses into blood circulating through vessels in the filaments and lamellae. Simultaneously, carbon dioxide in the fish's bloodstream diffuses into the water and is carried out of the body (Verheyen *et al, 1994).*

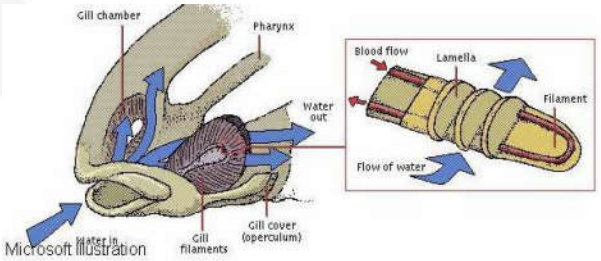

Fig. 1: Diagram showing the structure for respiration (gas exchange) in fish

Effects of Oxygen Levels on Oxygen Uptake by Fish

It is commonly thought that if there is not enough oxygen in the water, then the fish will be seen gasping at the surface but this is a last resort means to breathe. The first indication there may be a dissolved oxygen problem in the water is when the fish become unusually lethargic and stop feeding. As oxygen levels decrease, the fish do not have enough energy to swim and feeding utilises yet more oxygen. Most fish species will tolerate a drop below the minimum values for a short period of time, probably the cold water species are likely to tolerate a lower level than tropical fish (Jobling, 1995). However, the period of time during

which the oxygen level drops below the required minimum level, will cause the fish to become stressed resulting fish death. Some stress related diseases such as fin rot and white spot may also occur (Crampton *et al*, 2003).

Materials and Methods

Experimental Fish

This study was done by using Molly (*P sphenops*) in fresh water habitat. *P. sphenops* is a species of fish, of the genus *Poecilia* under family Poeciliidae, known under the common name molly; to distinguish it from its congeners, it is sometimes called short-finned molly or common molly. Poeciliidae is a family of freshwater fishes of the order Cyprinodontiformes, the tooth-carps, and include well-known live-bearing aquarium fish, such as the guppy, molly, platy, and swordtail. The wild-type fish are a dull silvery color, often sprinkled black all over. The common molly can produce fertile hybrids with many *Poecilia* species, most importantly the sail fin molly. The male black mollies generally tend to be mildly aggressive. Mollies rank as one of the most popular feeder fish due to high growth rate, birth size, reproduction, and brood number. In the case study, 1000 molly of 0.5 - 1 g. in weight were reared in replica at 40%-100% DO levels in 20 different aquarium of 2′ X 1′ X 1′.

Experimental Design

The fish were exposed to different levels of oxygen saturation. The system consists of 20 different aquarium of 10 liter capacity. Each aquarium contains 50 experimental fish. Every alternate day 5 gms of food was given. The DO was regularly measured by Winkler's method (1888). The subsequent effect of oxygen saturation levels on growth and feed conversion ratios were taken in three, six, nine and twelve months.

Sampling and Measurements

The body mass and population growth performance were measured every two weeks, while oxygen saturation, temperature and salinity were recorded daily. Population rate of the experimental fishes of each aquarium were taken in every two weeks to obtain the Specific Population Growth Rate (SPGR). Both the initial and final number of total fish population were used to calculate in terms of SPGR. The fish were fed manually in every alternate day. Approximately 5 gms of food were given every times. The Food Conservation Ratio is calculated as: FCR = Total amount of feed consumed/increase in population during the same time.

Result

The results showed that oxygen saturation level had a great effect on the reproduction, growth and food conversion ratio. Below 60% of the normal DO, the population growth was comparatively lower. Feed conversion ratio was higher at 60% and above, compared to lower oxygen level. However, it is found that at highest DO level the body mass increase is little lower than medium DO level (75.1%). The rate of population increase is highest at DO 88 % but the body mass increase is little lower (91%) in that DO.

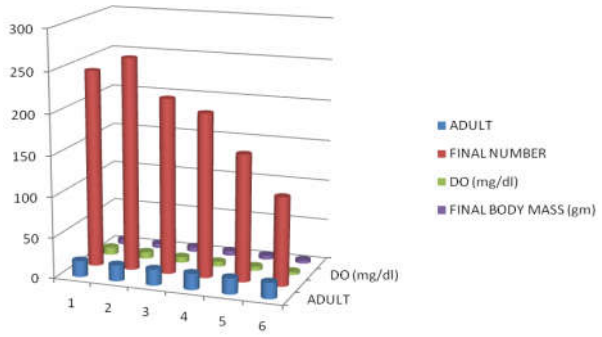

Fig. 2: Relation between rate of increase of number of fish, DO and body mass

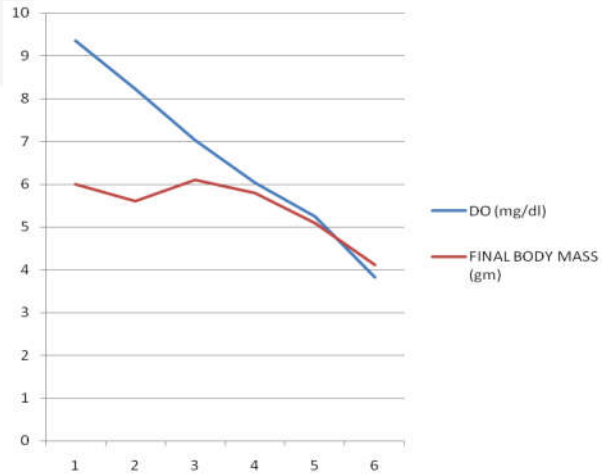

Fig. 2: Relationship of body mass increase and the DO level of water
(Y axis = Percent of dissolved oxygen, 1 unit equivalent to 10%
X axis = final body mass in gram weight)

Discussion

The results of the experiment under different oxygen levels clearly showed that growth is affected

by the level of oxygen saturation. During this period, the SPGR was highest at 88% saturation. The best FCR was obtained in the groups with highest growth rate although there was no significant ifference in FCR of fish reared at different oxygen saturation levels. However, this species appear to be more sensitive to oxygen saturation that increases its growth rate with increasing saturation up to 100%. It is recommended that in Tropical freshwater fish- 5 mg per litre (80% saturation) is necessary and most effective. Mallya (2007) showed that oxygen saturation level had a positive effect on the growth and feed conversion ratio when it was set at 80%-120% saturation. At 140% the growth was slightly lower and the feed conversion ratio was higher at 60% and 140% compared to the other groups. The conclusion was that oxygen saturation level has an effect on growth and feed conversion ratios of fish, and in the case of Atlantic halibut, the growth rate is higher when the oxygen level is between 80% and 120%. The feed conversion ratio for halibut was lower at 120% oxygen saturation.

Conclusions

The results suggest that oxygen saturation levels affect both growth performance and feed conversion ratios of fresh water molly. The maximum population growth rate and lowest feed conversion ratio in this species can be attended at higher oxygen saturation levels between 90% and 120%. However, more research is needed in order to know at which saturation point the population growth is maximized.

Acknowledgement

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Original Article

Economics of Fish Production at Kalna and Its Adjacent Areas, Burdwan District, West Bengal, India

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Abstract

Culture of fish involves various processes like preparation and management of pond, liming, removal of weeds and weedivorous fish, nurturing the desirable species in a scientific manner and harvesting the fish. Profitability depends upon proper management of all the operations involved in the fish cultural activities. It also depends on factors like productivity, cost of production, and market price of the product. Minimization of cost and maximization of productivity help to improve the profitability. This paper highlights the production, productivity and profitability of fisheries in and around Burdwan based on the results of a primary survey conducted among the selected fishermen household units (Samudragarh (23°34'N and 88°32'E); Dhatrigram (23°27'N and 88°31'E) and Kalna (23°22'N and 88°34'E).

Keywords: Fish Culture; Production; Profitability Analysis; Burdwan; West Bengal; India.

Introduction

According to 2011 census, India's population increases 181 million people from 1.03 billion in 2001 to 1.21 billion in 2011. It is expected that India will become the most populous country in the world by 2030 overtaking China creating food scarcity and huge amount of unemployment. West Bengal is the fourth populous state in India after Uttar Pradesh, Maharashtra and Bihar. As per census 2011 estimates, the state has population of 9.13 crore accounting for 7.55 percent of India's population. Burdwan is a key district in West Bengal from the human resource growth perspective. As per 2011 census, the district has a population of around 77.24 lakhs, making it the third most populations in the state. The district has a relatively high proportion of urban population, accounting for 39.87 percent of total population. Burdwan's population density at 1100 persons per sq km is marginally higher than state average of 1029 persons per sq km. The adult gender ratio in the district has increased from 922 females per 1000 males in 2001 to 943 females per 1000 males in 2011. Around 33.40 percent of total district population comprised of reserved categories. Burdwan has a literacy rate of 77.15 percent (Ramsundar, 2011).

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Plant proteins are deficient in certain essential amino acids notably methionine, tryptophan and lysine which are essential for healthy growth while, animal proteins are rich in these amino acids and are therefore described as first class or good quality protein (Adeniyi et al., 2012; Dalhatu and Ala, 2010). Fish is an important source of high quality protein, providing about 16% of the animal protein consumed by the world's population (FAO, 1997). Asides from being a source of protein for livestock, fish plays an important role medicinally as it replenishes the human body with vitamins A and D; calcium, phosphorus and lysine; sulphur and amino acids (Ohen & Abang, 2007). Fish allows for protein improved nutrition, in that it has a higher biological value in terms of high protein retention in the body

(Anthonio & Akinwumi, 1991), higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein. World aquaculture output has increased substantially, from less than 1 m t of annual production in 1950 to 63.6 m t in 2011, increasing at three times the rate of world meat production (2.7% from poultry and livestock together) in the same period (FAO, 2012). About 68% of total population spends their livelihood from agriculture and per capita income is very low. Fisheries can play important role in this aspect. Realizing its importance during the fifth-five year plan the Government of India introduced beneficiary-oriented programme in the form of a pilot project entitled 'Fish Farmers Development Agency' (FFDA) to provide self employment, financial, technical and extension support to fish farming in rural areas. In 1974-75 this programme was further extended under World Bank Assisted, Inland fisheries project to cover about 200 districts of various states in India. The nutritional requirement is particularly crucial in a developing country like India where malnutrition and starvation are the major problems faced by millions of rural dwellers.

In India, about 14 million people are employed in fisheries sector either directly or indirectly (NCAP, 2008). It contributes about 1.5% to total GDP (Gross Domestic Product) and about 5.2% to the agricultural GDP. India witnessed an impressive growth in inland fisheries and the past ten years has witnessed both horizontal and vertical expansion, with total inland fish production increasing from 2.84 mt in 2000– 01 to 4.86 mt in 2009-10, an increase of over 70% (Indiastat, 2011).

Fish is one of the most favorite food items of Bengali. Out of the total net area under effective pisciculture 1, 43,336.66 ha in West Bengal, the district Burdwan covers 23,313 ha net area under effective pisciculture (Table 1). Pisciculture is a key allied activity in the district with a workforce of over 1.4 lakhs involved in these activities. Large number of tanks and ponds are stocked every year with fry and fingerlings not following scientific methods. Use of scientific methods in fish culture and proper management of fish culture activities offer immense scope for improvement the productivity and profitability.

Fish production depends upon various factors, such as pond preparation, control of weeds, control of predator, liming, proper stocking, monitoring of physicochemical parameters, use of manure, supplementary feeding, netting, stock manipulation, harvesting. The following factors are considered at the time of selecting ponds for cultivation: suitable shape of the pond, assured supply of adequate quantity of water, quality of soil and water of the pond, road connection for transportation of fish, pond location must be free from social problems such as poaching, malicious damage etc.

This paper highlights the production, productivity and profitability of fisheries based on the results of a primary survey conducted among the selected fishermen household units (Samudragarh (23°34'N and 88°32'E); Dhatrigram (23°27'N and 88°31'E) and Kalna (23°22'N and 88°34'E) in the district Burdwan.

Table 1: Pisciculture Scenario

State/ District	Net Area Available (ha.)	Net area under effective pisciculture (ha.)	Number of people employed
West Bengal[s]	5,93,512.4	1,43,336.66	21,63,062
Burdwan[d]	31,181	23,313	1,43,950

Materials and Methods

Primary data have been collected through administering questionnaires among 240 selected fishermen household units of 12 villages (20 units from each village). However, analysis has been made according to analysis method adapted by Dandapat and Islam, 2009; considering 200 questionnaires as 40 questionnaires have been rejected due to incomplete responses. We also collected the data of fish collected by fishermen.

Data were collected for a period of two years (2013-2014 & 2014-2015). Collected data have been tabulated and analyzed using simple statistical analysis t test.

Results

During the period of survey, we have noticed six species of cultivable carps namely Major carps (Rohu-*Labeo rohita*, Catla- *Catla catla*, Mrigel- *Cirrhinus mrigala*), Exotic Carp (Grass carp-*Ctenopharyngodon idella*, Silver Carp- *Hypophtahlmichthys molitrix*, Common Carp-*Cyprinus carpio*). Besides this we also noticed varieties of fishes out of which most dominant

and prevalent fish species are *Puntius ticto* and *Channa punctatus* which are of economic importance and high protein content.

At the time of survey we have also noticed that in composite fish culture stocking density were maintained (Table 2).

Profitability Analysis

Culture of fishes involves important cost elements:

i. Annual Non- Recurring Costs (Rent of Ponds, Cost of preparation and maintenance of pond).

ii. Recurring costs (cost of weed clearance, fish eradication, labor cost, cost of organic/inorganic manure, cost of seeds of fishes/ fry, cost of harvesting, cost of netting (insect removal), transport cost

iii. Other costs such as depreciation on loan, interest on loan etc.

Also Profitability Depends on Three Stages of Fish Production

i. Raising of fry from Nursery Pond

ii. Raising of fingerling from Rearing Pond

iii. Raising of Table Size Fish From culture

The fishermen were interviewed to know their cost structure relating to above three stages of fish culture. The figures have been derived on the basis of their responses, because most of the fishermen usually do not maintain systematic account. Information on sale price has been obtained from wholesale market and average price has been taken into consideration. The

Table 2: Stocking density observed in composite fish culture

Species of fish	% composition
Major Carps	
Rohu	25
Catla	10
Mrigel	10
Exotic Carps	
Grass Carp	10
Silver Carp	25
Common Carp	20

Table 3: Profitability of Raising Fry from Nursery Pond

A. Annual Non-Recurring Cost	Annual Cost (Rs.)
Annual Rent of pond (1 bigha/0.133 hectare)	12000
Pond Preparation and maintenance	5000
Total	**17000**
B. Recurring Cost	
Labour charges	7000
Weed clearance	1000
Insect Removal (netting)	750
Organic manure	2000
Cost of fish seed (4 lakhs pieces)	8000
Transport	2500
Cost of feed	5000
Harvesting	4000
Depreciation of net	500
Total	**30,750**
Total cost (A+B)	**47,750**
C. Income	
Income from sale of 200000 fry at Rs. 450/- per 1000 pieces [Expected Fry Production at an average rate of survival of 50% of 4 lakhs pieces]	90,000
D. Net Profit [C-(A+B)]	**42,250**

Table 4: Profitability of Raising Fingerling from Rearing Pond

A. Annual Non-Recurring Cost	Annual Cost (Rs.)
Annual Rent of pond (1 bigha/0.133 hectare)	14,000
Pond Preparation and maintenance	6000
Total	**20,000**

B. Recurring Cost	
Labour charges	9000
Weed clearance	1200
Insect Removal (netting)	1000
Organic manure	2400
Cost of fry (15000 pieces)	10000
Transport	2500
Cost of feed	8000
Harvesting	5000
Depreciation of net and contingency	500
Total	**39,600**
Total cost (A+B)	**59,600**
C. Income	
Income from sale of 10500 fry at Rs. 9,000/- per 1000 pieces [Expected Fry Production at an average rate of survival of 70% of 15000 pieces]	94,500
D. Net Profit [C-(A+B)]	**34,900**

Table 5: Profitability of Raising of Table size fish from Composite Culture Pond

A. Annual Non-Recurring Cost	**Annual Cost (Rs.)**
Annual Rent of pond (1 bigha/0.133 hectare)	14,000
Pond Preparation and maintenance (MOC + Earth filling)	7,000
Total	**21,000**
B. Recurring Cost	
Labour charges	9000
Organic manure	2500
Cost of fingerling (1500 pieces)	22500
Transport	2500
Cost of supplementary feeding	5000
Harvesting	5000
Depreciation of handi and contingency	600
Total	**47,100**
Total cost (A+B)	**68,100**
C. Income	
Income from sale of 450 kg fish at Rs. 250/- per kg [Expected Fish Production at an average rate of survival of 90% of 1500 pieces and an average size of 2 kg]	1,12,500
D. Net Profit [C-(A+B)]	**44,400**

Table 6: Total Income, Expenditure and Savings of Fishermen Household units

	Rs
Net Profit per bigha from Nursery pond (Table 3)	42,250
Net Profit per bigha from Rearing pond (Table 4)	34,900
Net Profit per bigha from Composite Culture pond (Table 5)	44,400
Total Profit from three bigha	**1,21,550**
Average Income per household unit Per Annum (on the basis of average pond holding of three bigha)	1,21,550
Average household expenditure (8000/- per month × 12 months= 96000)	96,000
Savings per household unit per annum	**25,550**

cost structure and profitability of three different stages of production have been shown in Table 3, 4 and 5. Total Income, Expenditure and Savings of Fishermen Household units is presented in Table 6.

From the survey of 200 fishermen household units, it has been found that average pond holding per household unit is three bigha and average household expenditure Rs 8000/- p.m. Therefore, the average surplus per household unit is only Rs 25,550/- p.a. and Rs 2129.17/- p.m.

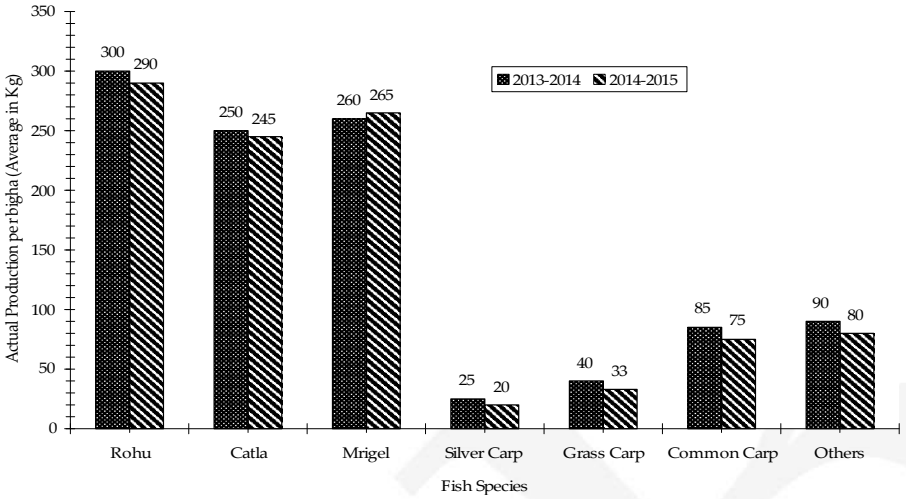

Fig. 1: Average production of fish

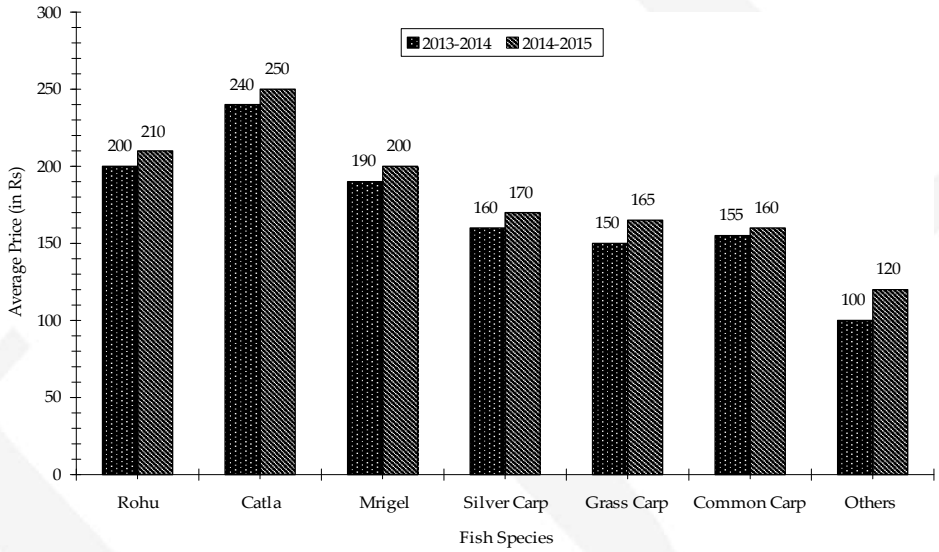

Fig. 2: Average annual selling price of fish (Rs. per Kg)

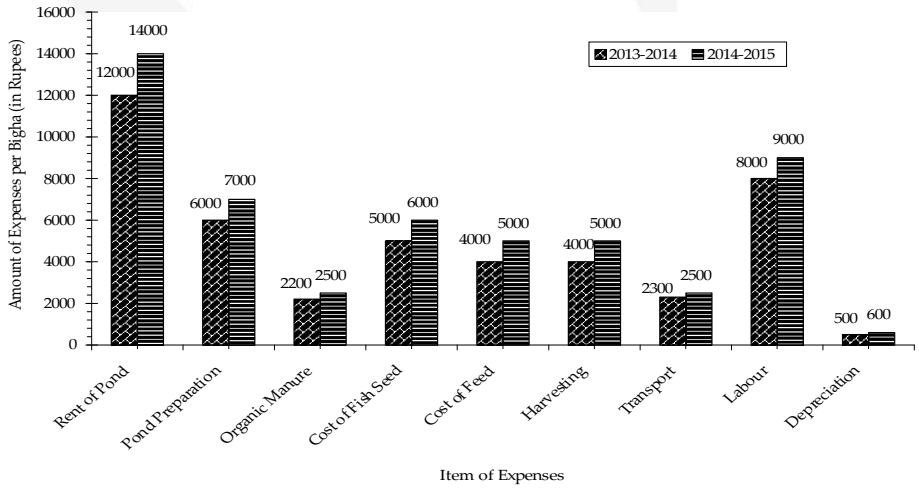

Fig. 3: Average Expenditure for Fish Cultivation

Statistical Analysis

In order to analyze the survey results and to examine relationship, if any, among average production of fish, average selling price and average expenditure of fish cultivation data were collected.

Student t test has been applied to find out the relationship among average production of fish (Figure 1), average selling price of fish (Figure 2) and average expenditure of fish cultivation (Figure 3).

Hypothesis:

H_0: There is no significant difference between:

i. The average production between two years

ii. The average selling price of fish for two years

iii. The average expenditure of fish cultivation for two years

H_A: Difference is Significant in the above Cases

In case of average production of fish, the calculated value of t (2.965) is greater than the tabulated value (2.45) at 5% level of significance. Therefore, H_0 cannot be accepted. So it can be said that the average production of fish for two years have been significantly different.

In case of average selling price, the calculated t value (6.358) is greater than the tabulated value (2.45) at 5% level of significance. Therefore, H_0 cannot be accepted. So it can be said that the average selling price of fish for two years have been significantly different.

In case of average expenditure for fish cultivation the calculated value of t (4.343) is less than the tabulated value (2.31) at 5% level of significance. Therefore, H_0 in this case is accepted.

So, it can be said that the average expenditure of fish cultivation in two years have not been significantly different.

Discussions

A serious lacuna in the country is availability of economics of respective species culture data. For any business planning or growth analysis, current or the latest data is necessary and unfortunately an organized/authentic data assessment system does not exist. In addition, business planning and market assessment would not be effective on old data because of the rapidly changing aquaculture scenario. In the recent years, The Indian economy, inflation rate has been increased gradually and cost of ingredients and fertilizer are also increased. This intern affected the cost of production or profit in fish culture practices. But, we have very limited economic information is available for carp seed rearing and farming. Therefore, we have conducted the survey on economics of seed rearing and farming of carps with reference to basic input. This was done for economic efficiency with combination of technical and allocative efficiencies.

The monthly and annual income of fishermen household units is very poor. They have not adequate fund for fish cultivation and so they have to lend money from Mahajans, who lend them money at very high rate of interest. Higher rate of interest, and lower price for fish production bound the fishermen to commit suicide or to live in debt traps of Mahajans.

5. Suggestions to improve production, productivity and profitability of fisheries

1. A well coordinated and collective effort made to develop fish-cooperatives for all-around development of production of fish.
2. Water areas above one acre may be incorporated under control of cooperative society.
3. Scientific techniques must be adapted in order to increase production.
4. Regional Offices of different National Institutes of Fisheries should be set up.
5. Fishermen must be encouraged to establish aqua club that may facilitate developing awareness about fisheries.
6. Knowledge about different fish disease is necessary to farmers.
7. Proper storage and processing facilities is must.
8. Fish farming in the area is dominated by males; females should be encouraged to participate in fish farming as a means of augmenting their income.
9. Unemployed youths in the study area should be trained in fish farming production methods and given loans to engage in fish farming business which is a very profitable enterprise in the area with high rate of return per capital invested.

Conclusions

Based on the results of these experiments, it can be concluded that though the fish seed rearing and farming is profitable, the margins are very narrow. Since the input costs and labor costs are increasing significantly, one must know the availability resources, capital and the projected profit before starting of the fish farming activity.

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Original Article

Optimization of Carbon Sources for the Amylase Production and Growth of *Bacillus licheniformis* JAR-26 under Submerged Fermentation

Nand Lal*, Jeevan Jyoti*,, Priti Sachan***

Abstract

Amylases (EC 3.2.1.1, 1, 4-α-D-glucan glucanohydrolase) are one of the most important and oldest industrial enzymes that hydrolyze starch at α-1, 4 glycosidic bond in the interior of the starch molecule, and hold the maximum market share of enzyme sales. Amylases are ubiquitously produced by plants, animals and microorganisms, however, microbial sources are the most preferred for large scale production and industrial use. The production of α- amylases from microbes depends on the strain, physical (pH, temperature, aeration) and nutritional (carbon, nitrogen, mineral ions) factors. Keeping this in view, the present study aimed to investigate effect of different concentrations (control, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% w/V) of five carbon sources *viz* starch, glucose, maltose, fructose and sucrose on growth and enzyme production from *B. licheniformis* JAR-26. Among the tested carbon sources, maltose proved best carbon source for amylase production and maximum amylase production was recorded at 2% maltose (4.181 U/ml of medium). At this concentration growth/OD was 1.529 and bacteria could utilize 98.2% of the available sugar in the medium. After maltose, starch was second suitable source for enzyme production and 2% starch showed 3.622 U/ml enzyme production. Glucose and fructose resulted in higher biomass yield (maximum biomass at 2% glucose, OD=1.741) in comparison to other sources but amylase production was very poor (lowest at 4% fructose, 0.710 U/ml). From comparison of the various treatments, it is suggested that for maximum biomass production of *B. licheniformis* JAR-26, growth medium may be supplemented with 3% glucose whereas to achieve maximum amylase production culture medium may be supplemented with 2% maltose under submerged fermentation.

Keywords: Amylase Production; Submerged Fermentation; *Bacillus licheniformis*; Carbon Source; Maltose.

Introduction

Amylases are widely used starch hydrolyzing enzymes with the maximum market share (about 30%) of the total enzymes sales. α-Amylases (EC 3.2.1.1; α-4-glucan glucano-hydrolase) are calcium containing endoamylases catalyze hydrolysis of starch and related carbohydrates by randomly cleaving internal α-D (1-4) glycosidic linkage, yielding glucose, maltose, maltotriose, and other oligosaccharides (Ryan, 2011). Although amylases can be obtained from higher plants and animals also, the enzymes from microbial sources, particularly grown in extreme environments, prove useful for industrial processes/demand due to its widespread use in the food, brewing, textile, detergent and pharmaceutical industries. Moreover, with the advances in biotechnology, the amylase application has expanded in many fields such as clinical, medicinal and analytical chemistry in addition to widespread use in starch saccharification and distilling industries.

The major advantage of using microorganisms for the production of amylases is continuous economical bulk production and their relatively easy genetic

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manipulation to obtain enzymes of desired characteristics. A significant increase in amylase production and utilization occurred in early 1960s with the advent of *Bacillus subtilis* α-amylase and *Aspergillus niger* glucoamylase for the production of dextrose from starch as alternative to acid hydrolysis. Today, a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Several *Bacillus* species (*Bacillus licheniformis*, *Bacillus amyloliquefaciens* and *Bacillus stearothermophilus*) are among the versatile producer of α-amylases (Naidu and Sharanraj, 2013). The two bacterial strains *Bacillus amyloliquefaciens* and *Bacillus licheniformis* have been exploited on the industrial scale (Alariya *et al.,* 2013). Production of amylases is influenced by physical (pH, temperature, aeration etc.) and nutritional factors (carbon, nitrogen, mineral ions etc.) which control the growth and metabolism of producer organism (Halder *et al.*, 2014; Abel-Nabey and Farag, 2016). Nutritional factors in particular play vital role for the commercial production of bacterial amylases (Dutta *et al.,* 2016). The nature and concentration of carbon and nitrogen source in the culture medium are reported to important factors governing bacterial growth and amylase production (Akeel and Umar, 2010; Lal *et al.,* 2016).

Soils receiving the biodegradable kitchen wastes are one of the rich sources of starch degrading microorganisms as they are rich in starchy substances. *B. licheniformis* JAR-26 is an acidophilic and thermostable extracellular α-amylase producing acidophilic bacteria isolated from soils rich in spoiled tomatoes as kitchen waste (Jyoti *et al.,* 2011). Keeping in view the role of nutritional factors on bacterial growth and amylase production, the present study aims to investigate the effects of various concentrations (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0% w/V) of different carbon sources namely Starch, Glucose, Maltose, Fructose and Sucrose on amylase production, growth and sugar utilization by *Bacillus licheniformis* JAR-26.

Material and Methods

Microorganism

Starch hydrolyzing *Bacillus licheniformis* JAR-26 was isolated from spoiled tomatoes and collected in sterilized stoppered glass vials.

Media and Chemicals

Starch, Yeast extract, Peptone, $MgSO_4.7H_2O$, KH_2PO_4, NaCl, $CaCl_2$, Agar, Distilled H_2O, Phosphate buffer, Iodine solution, 3, 5 dinitrosalicylic acid (DNS), Glucose, Maltose, Fructose, Sucrose, Sephadex G-100, DEAE-Cellulose (DE-52), CM-Cellulose, Acrylamide, Bis-acrylamide, N,N,N'n'-tetramethylethane-1,2-diamine (TEMED), Sodium dodecyl sulphate (SDS), Ammonium persulphate.

Isolation of Microorganism

The thermostable, acidophilic starch hydrolyzing bacteria (*B. licheniformis* JAR-26) was screened for extracellular acidophilic amylase production by using starch medium containing (g/L): Starch (Merck, Germany), 10.0; yeast extract, 5.0; peptone, 2.0; $MgSO_4.7H_2O$, 0.5; KH_2PO_4, 0.5; NaCl, 1.5; $CaCl_2$, 0.1; Agar, 20.0. Initial pH was adjusted to 5.5. One gram of each sample was suspended in 9.0 ml of sterile water and 0.1 ml of suitably diluted suspension was spread on the agar plates. The plates were incubated at 45, 50, 55 and 60 °C for 24 to 48 h. The isolated colonies were flooded with iodine solution and colonies bearing good colorless halos around them were picked and maintained on starch agar slants at 4 °C and further assessed for enzyme production in liquid medium. The characterization and identification of the isolate was made following Bergey's Manual of Systemic Bacteriology. The method of identification used was as given by Collee *et al.* (1996).

Amylase Production

The basal fermentation medium for enzyme production contained (g/L): Starch, 10.0; yeast extract, 5.0; peptone, 5.0; KH_2PO_4, 0.12; $CaC1_2.2H_20$, 0.12; $MgSO_4.7H_20$, 0.12; $MnSO_4.4H_20$, 0.02. Initial pH of the medium was adjusted at 5.5 and 50 ml of medium in 250 ml of Erlenmeyer flasks were inoculated with a cell suspension of optical density 0.5 (prepared from 24 h old culture). All the flasks were incubated for four days on a rotary shaker (Remi) at 170 rpm at 45 °C. Samples were drawn after a time interval of 12 h, centrifuged at 8000 Xg for 10 minutes and cell free culture supernatant fluid was used as enzyme source.

Assay of Enzyme

Culture filtrate (Supernatant) was used for assessing enzymatic activity by the method of Srivastava and Baruah (1986). One ml of 1% (w/V) starch (Merck, Germany) solution was taken in test tube and 0.2 ml of 0.2 M phosphate buffer (pH 5.5) and 0.2 ml of deionized water was added to it. The

mixture was equilibrated at 70 °C for 10 minutes in a water bath. 0.1 ml of supernatant was added and then reaction was stopped by adding 1.0 ml of 3, 5 dinitrosalicylic acid (DNS). The mixture was heated and the color intensity was measured at 540 nm (Bernfield, 1955) using a spectrophotometer (Systronics Spectrophotometer 169). One unit of amylase activity was defined as the amount of amylase that liberates 1.0 mg of glucose per minute under assay conditions. In all the above experiments the enzyme activity was calculated as the average of three independent sets of experiments (the standard deviation in all cases was found negligible).

Effect of Carbon Source

The basal fermentation medium was supplemented with the different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0% w/V) of carbon sources i.e. starch, glucose, maltose, fructose and sucrose and its effect was recorded on bacterial growth, amylase production and sugar utilization by test bacteria.

Results and Discussion

The test organism was isolated from spoiled tomatoes and screened by zone hydrolysis method (Figure 1) and later identified as *Bacillus licheniformis* JAR-26 (Figure 2) according to Bergey's Manual of Determinative Bacteriology (Jyoti *et al.*, 2011).

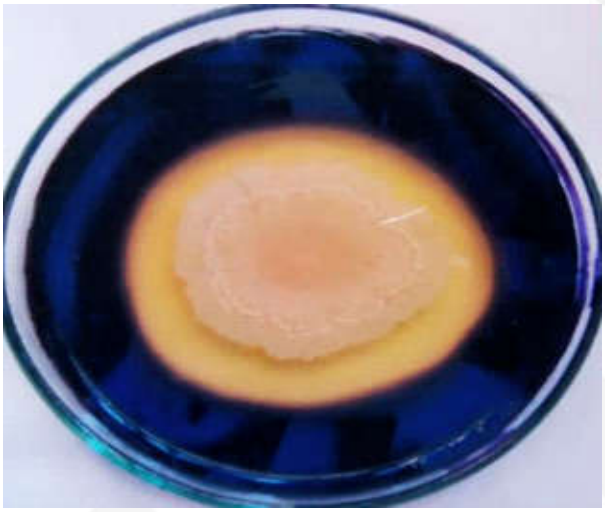

Fig. 1: Starch Hydrolysis by isolate JAR-26

Fig. 2: *Bacillus licheniformis* JAR-26

Carbon forms backbone of all biomolecules (protein, lipid, carbohydrates, nucleic acids etc.) and is required relatively in large amounts than other nutrients in the production medium. Starch is most widely accepted carbon nutrient source for induction of microbial amylolytic enzymes, hence, 1% (w/V) starch was considered as reference and essential component of basal fermentation medium. Effects of different carbon sources (starch, maltose, glucose, sucrose and fructose) and their concentrations on α-amylase production, growth and sugar utilization by *Bacillus licheniformis* JAR-26 are summarized in Figure 3, 4 and 5, respectively, and all the parameters

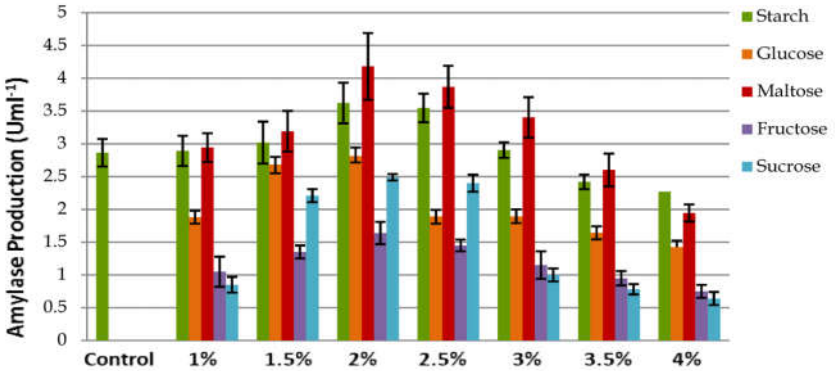

Fig. 3. Effect of different concentrations of carbon source(s) on Amylase Production by *B. licheniformis*

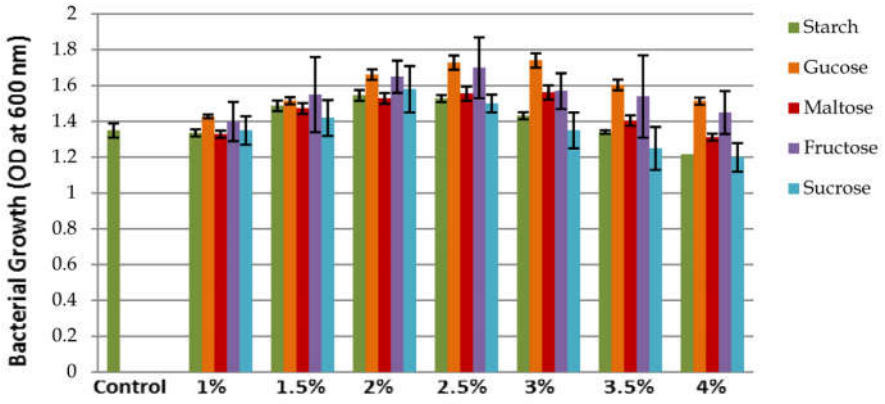

Fig. 4. Effect of different concentrations of carbon source(s) on growth of *B. licheniformis*

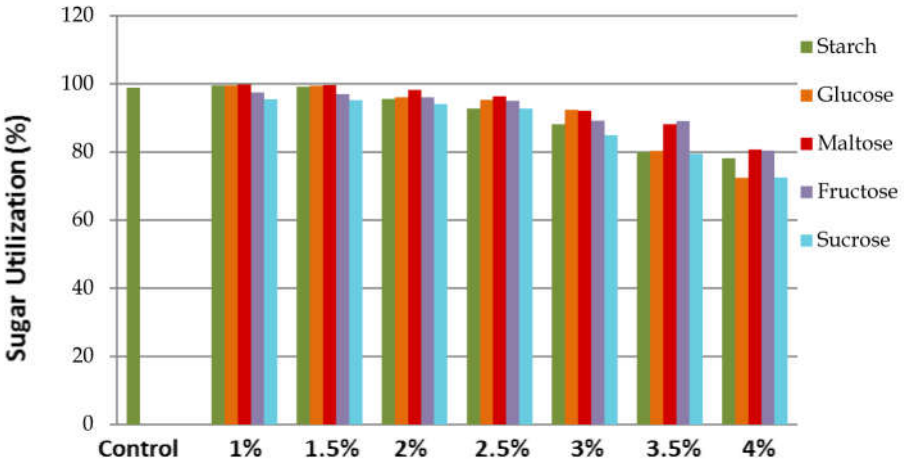

Fig. 5. Effect of different concentrations of carbon source(s) on sugar utilization by *B. licheniformis*

varied with the nature and concentration of carbon source used in the medium.

As shown in Figure 3, none of the tested carbon source enhanced the enzyme production by *B. licheniformis* except maltose and starch over control medium. Even amylolytic activity was inversely affected by glucose, fructose and sucrose with the amylase production and was recorded lower than control. Maltose was found to be the most suitable carbon source for amylase production and maximum amylase production was achieved at 2.0% Maltose (4.181 Uml^{-1}). At 2.0% Maltose, growth was 1.529 in terms of OD at 600 nm and bacteria could utilize 98.2% of available sugar in the medium. Similar findings are reported by Thippeswamy *et al.* (2006) in *Bacillus* species (B_3) where highest amylase production (0.464 Uml^{-1}) was induced by maltose. Suribabu *et al.* (2014) also found maltose superior than other carbon sources tested for amylase production with *Brevibacillus borostelensis* R1 under submerged fermentation.

Starch proved to be second best carbon source for amylase production by *Bacillus licheniformis* JAR-26. Starch at 2% showed 3.622 Uml^{-1} amylase productions and growth was 1.546 in terms of OD at 600 nm and the bacteria could utilize 99.6% of available sugar in the medium. Starch is a generally accepted nutritional component for induction of amylolytic enzymes. Bajpai and Bajpai (1989) observed enhanced production of α-amylase when *Bacillus megaterium* grown on starch containing medium. Mishra and Behera (2008) also reported 2% starch concentration as suitable carbon source for amylase production as well as bacterial growth in *Bacillus* species.

It can be inferred from the Figure 4 that Glucose and Fructose resulted in higher biomass yields, 1.75 at 3.0% Glucose and 1.70 at 2.5% Fructose, respectively, which was superior over the values observed in the present study for Maltose and Starch. On the contrary, amylase production was too poor (2.814 Uml^{-1} & 1.64 Uml^{-1}, respectively) with glucose and fructose. Haseltine *et al.* (1996) during studies on hyperthermophilic archaebacteria *Sulpholobus*

solfataricus reported that glucose represses amylase production and inhibits expression of amylase gene and same seems to be true for *B. licheniformis* JAR-26. Sucrose turned to be least preferred carbon source for *B. licheniformis* JAR-26 for all the three parameters i.e. amylase production, growth and sugar utilization except 2.5% where amylase production exceeded over corresponding glucose and fructose concentrations. Utilization of sucrose by bacteria is routed through its conversion into glucose and fructose and among these two monosaccharides glucose is the most preferred substrate. Equal concentrations of glucose and sucrose did not show corresponding performance as the glucose produced from sucrose by hydrolysis remains in lesser amounts. Fructose turned closer to glucose and exhibited nearly similar effects/trends on growth and sugar utilization but for amylase production sucrose turned superior over fructose in the range of 1.5 to 2.5 % (w/V). This observation on fructose and sucrose for amylase production is in conformity with the observations of Suribabu *et al.* (2014) on *Brevibacillus borostelensis* R1. In a study on *B. flavothermus* by Kelly *et al.* (1997), the presence of sucrose, fructose and glucose in the media resulted in good bacterial growth with little or no amylase production. On contrary to findings of present study, Sreekanth *et al.* (2013) reported 2% glucose to increase amylase activity significantly (66 Uml^{-1}) among various tested carbon sources.

Sugar utilization by *B. licheniformis* in the presence of various tested carbon sources is shown in figure 5 and varied for bacterial ability to utilize carbon in different forms. It has been reported that the synthesis of carbohydrate degrading enzymes including amylase (s), in most of the microbial species in general and the genus *Bacillus* in particular, is subjected to catabolic repression by readily metabolisable substrates such as glucose and fructose (McMahon *et al.*, 1999; Suman and Ramesh, 2010) like most other inducible enzymes and the same turns to be true in present study.

Conclusion

The production of amylases by microbes is known to be affected by a variety of physiochemical factors i.e. composition of the growth medium, inoculum age, pH, temperature, carbon and nitrogen source and mineral elements. From the present study it can be concluded that the bacterial isolate *B. licheniformis* JAR-26 produces significant amount of amylases (4.181 Uml^{-1}) at 2% maltose concentration in culture medium. Starch proved to be second most suitable carbon source with 3.662 Uml^{-1} amylolytic activities at 2% concentration. The role of glucose, fructose and sucrose was found less significant than other carbon sources (maltose and starch).

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Review Article

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Bird Watch at Jalangi: Avian Diversity and Seasonal Abundance within the River Jalangi, Nadia (WB)

Monojit Ray

Abstract

Though rivers are not the great place for bird watch, specially for migratory birds, still the river Jalangi is different from other rivers of West Bengal with respect to avian diversity. The present article deals with the avian diversity and seasonal avifaunal abundance at the Jalangi river within Nadia district (WB) during April 2014 to March 2015, in which more than fourty five species of birds belonging to different families were recorded. Maximum species were sighted during winter season, some birds were found to be migratory, some birds were residential migratory and some other resident. Variation in food availability in different seasons affects on avifaunal diversity in study area and the variation of food availability is controlled from behind by the factors like rate of photosynthesis within river, soil fertility, physicochemical parameters, ion concentrations of river water etc. The avifauna is important for the ecosystem as they play various roles as scavenger, pollinators and predators of insect and pest.

Keywords: Birds; Jalangi; Diversity.

Introduction

The river Jalangi flows 206 km through the Nadia district from the direction of north-east to the south-west. Jalangi meets the river Bhagirathi near Nabadwip Town (23.252 N 88.222 E), Nadia. The Jalangi river water sources are majorly river Bhairabs water and underground water. The river water flows from the direction of Bhairab to Bhagirathi. The river is the habitat of various aquatic flora and fauna. The entire biosphere within the river Jalangi depends on the physico-chemical parameters of the river water. Domestic use, Irrigation, soil erosion from bank for brick factory, water transportation, "bisarjan" of gods clay models, swage water from towns and villages and jute stem ratting etc. are the major source of the pollutants in Jlalangi. The physico-chemical parameters, specially BOD and COD values of the river water remain between 1 - 6 mg/liter and 7 -16 mg/liter respectively. These reflects the low level of water pollution. Throughout the year the river jalangi water remain slightly alkaline, moderately hard. The dissolved oxygen value lies between 6.1 – 8.1 mg/liter (Table 1). Round the year, the river water contains sufficient nitrate, phosphate, potassium, magnesium, calcium etc. biologically significant ions (Table 2). Huge amount of green algae, blue green algae, phytoplaktons, diatoms etc. grow up within the river. Hence the river is an ideal habitat of small fishes, pila, shrimps, frogs, insects etc. Which in-turn provide plenty foods for the residential as well as for the migratory birds. Flora and fauna specimen collections, bird watch were a continuous process from river banks and small boats at different locations. There are fourty seven type of birds present at the river Jalangi. Within these fourty seven types, five are migratory, fourteen are resident-migratory and other twenty eight are of resident type (Table 3).

The sodium, potassium, calcium, magnesium ion concentrations present in river water are sufficient to strengthen the lotic ecosystem. Phosphate ion concentration and nitrate concentrations are also

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sufficient for aquatic life. Sufficient bicarbonate concentration indicates the high rate of photosynthesis within river by the aquatic flora. This in turn, produce sufficient algae and vegetations i.e., foods for the fishes, insects, preys and birds. Calcium, phosphate, carbonate and sulphate ions are responsible for biomineralisation and sufficient crustaceans, snails etc., which are plenty within the river.

Discussion

The Physico-chemical parameter data (Table 1.) clearly indicates that the river water is moderately hard, slight alkaline and have sufficient dissolved oxygen for the survival of aquatic animals. Low turbidity reflects more penetration of sunlight, i.e., more photosynthesis by aquatic flora, hence, more food availability for fauna present. BOD and COD values clearly suggests the low level of pollutants within river. The pH of river water lies between 7.2 -8.35. The salinity value lies between 108–270 mg/liter suggests that, the river contain fresh water.

Considerable studies on avifaunal diversity from different freshwater wetland of India have carried out by many researchers buy yet no literature is available about river Jalangi, Nadia, West Bengal. This study is therefore beneficial document of the avifaunal diversity of the river Jalangi.

In the river Jalangi, available 47 type of birds, belong from fourteen orders and thirty family. The most common order is Passeriformes and fifteen type of birds belong from this order. The most common family are Scolopacidae and Ardeidae.

Among the charadriiforms present, Red-wattled Lapwing and Grey-headed Lapwing prefer insect and molluscus etc. Birds belong from Jacanidae family loves insect and other invertebrates from floting vegetation or the water surface. Birds belong from Scolopacidae family likes insects, small prey and crustaceans as food. Cormorants belong from Phalacrocoracidae family and they take small fishes (specially ell) and shrimps. Little Grabe belong from Podicipedidae family and grab small fishes. Egret and Herons present in the river belong from Pelecaniformes order and Ardeidae family. They consume small fishes, frogs, insects along with small crabs, grasshoppers and blue bottle flies etc. White breasted Waterhen, Common Moorhen and Common Coot are from the order Gruiformes and Rallidae family. Common Coot is carnivorous, whereas, Moorhen prefer wide variety of vegetable materials and small aquatic creatures. Asian Openbill feed mainly fresh water mussels and *Pilla sp.* Lesser Whistling Ducks are largely vegetarian, they also eat small fishes and snail etc. Coraciiformes (i.e., Kingfishers) feed mainly small fishes, shrimps and insects. White Wagtail and Large Pied Wagtail belong from Motacillidae family. Wagtails are insectivores. Shrikes belong from Laniidae family and feed mainly large insects, small birds etc. Black Drongo is a member of Dicruridae family, Prinia belongs to Cisticolidae family. Both of them consume mainly insects as food. Black-headed ibis or oriental white ibis, which have the conservation status "Near Threatened" were found a pair only. Black-headed ibis feeds on various fishes, frogs and other water creatures, as well as on insects.

Results

Table 1: Variation of Physico-chemical Parameters of River Jalangi during April 2014 to March 2015

pH	**7.20 - 8.35**
Conductance (µS/cm)	221 - 556
Hardness (ppm)	123.64 - 291.23
TDS (mg/Liter)	157 - 420
DO (mg/Liter)	6.1 – 8.1
Salinity (mg/Liter)	108 - 270
Turbidity (NTU)	3.5 – 6.1
Alkalinity (total) ppm	92 - 285
Alkalinity ($CO_3^=$) ppm	8 - 36
Alkalinity (HCO_3^-) ppm	84 - 265
BOD(mg/Lit) 3DAYS, 27°C	< 2 - 6
COD(mg/Lit)	7 -16

Table 2: Variation of biologically significant ion concentrations of River Jalangi during April 2014 to March 2015

HCO_3^- (mg/liter)	118.34 – 323.30
$CO_3^=$ (mg/liter)	4.8 – 21.6
Na^+(mg/liter)	8.28 – 24.37
K^+(mg/liter)	3.52 – 4.20
Mg^{2+}(mg/liter)	7.70 – 45.60
Ca^{2+} (mg/liter)	23 – 96.30
SO_4^{2-} (mg/liter)	< 2.5 – 6.3
PO_4^{3-} (mg/liter)	5.09 – 7.65
Cl^- (g/liter)	2.7 – 2.84
NO_3^- (mg/liter)	0.45 - 0.50

Table 3: List of avifaunal diversity of Jalangi River, Nadia District, West Bengal

No.	Order	Family	Scientific Name	Common Name	Habitat Status
1.	Charadriiformes	Scolopacidae	*Tringa nebularia*	Common Greensank	M
2.	Charadriiformes	Scolopacidae	*Tringa glareola*	Wood Sandpiper	M
3.	Charadriiformes	Scolopacidae	*Actitis hypoleucos*	Common Sandpiper	RM
4.	Charadriiformes	Scolopacidae	*Gallinago gallinago*	Common Snipe	RM
5.	Charadriiformes	Scolopacidae	*Tringa stagnatilis*	Marsh Sandpiper	M
6.	Charadriiformes	Jacanidae	*Hydrophasianus chirurgus*	Phesant tailed Jacana	R
7.	Charadriiformes	Jacanidae	*Metopidius indicus*	Bronzed winged Jacana	R
8.	Charadriiformes	Charadriidae	*Vanellus indicus*	Red-wattled Lapwing	R
9.	Charadriiformes	Charadriidae	*Vanellus cinereus*	Grey-headed Lapwing	R
10.	Suliformes	Phalacrocoracidae	*Phalacrocorax carbo*	Great Cormorant	RM
11.	Suliformes	Phalacrocoracidae	*Phalacrocorax niger*	Little Cormorant	RM
12.	Podicipediformes	Podicipedidae	*Tachybaptus ruficollis*	Little Grabe	RM
13.	Pelecaniformes	Ardeidae	*Ardea cinerea*	Grey Heron	RM
14.	Pelecaniformes	Ardeidae	*Casmerodius albus*	Large Egret	RM
15.	Pelecaniformes	Ardeidae	*Ardeola grayii*	Indian Pond Heron	R
16.	Pelecaniformes	Ardeidae	*Mesophoxy intermedia*	Median Egret	RM
17.	Pelecaniformes	Ardeidae	*Egretta garzetta*	Little Egret	R
18.	Pelecaniformes	Threskiornithidae	*Threskiornis melanocephalus*	Black-headed Ibis	RM
19.	Gruiformes	Rallidae	*Amaurornis phoenicurus*	White-breasted Waterhen	R
20.	Gruiformes	Rallidae	*Gallinula chloropus*	Common Moorhen	RM
21.	Gruiformes	Rallidae	*Fulica atra*	Common Coot	RM
22.	Ciconiiformes	Ciconiidae	*Anastomus oscitans*	Asian Openbill	R
23.	Anseriformes	Antidae	*Dendrocygna javanica*	Lesser Whistling Duck	M
24.	Cuculiformes	Cuculidae	*Centropus sinensis*	Greater Coucal	R
25.	Coraciiformes	Cerylidae	*Ceryl rudius*	Lesser Pied Kingfisher	R
26.	Coraciiformes	Alcedinidae	*Alcedo meninting*	Blue eared Kingfisher	R
27.	Coraciiformes	Halcyonidae	*Halcyon smyrnensis*	White-breasted Kingfisher	R
28.	Coraciiformes	Meropidae	*Merops orientalis*	Small Green Bee-eater	R
29.	Piciformes	Picidae	*Dinopium benghalense*	Lesser Golden Backed Woodpecker	R
30.	Bucerotiformes	Upupidae	*Upupa epops*	Common Hoopae	RM
31.	Passeriformes	Hirundinidae	*Hirundo rustica*	Common Swallow	RM
32.	Passeriformes	Laniidae	*Lanius cristatus*	Brown Shrike	M
33.	Passeriformes	Laniidae	*Lanius collurioides*	Burmese Shrike	M
34.	Passeriformes	Dicruridae	*Dicrurus macrocercus*	Black Drongo	R
35.	Passeriformes	Sturnidae	*Sturnus contra*	Asian Pied Starling	R
36.	Passeriformes	Sturnidae	*Acridotheres ginginanus*	Bank Myna	R
37.	Passeriformes	Corvidae	*Corvus splandens*	House Crow	R
38.	Passeriformes	Corvidae	*Corvus macrorhynchas*	Jungle Crow	R
39.	Passeriformes	Cisticolidae	*Prinia inornata*	Plain Prinia	R
40.	Passeriformes	Muscicapidae	*Copsychus saularia*	Oriental Magpie Robin	R
41.	Passeriformes	Motacillidae	*Motacilla alba*	White Wagtail	RM
42.	Passeriformes	Motacillidae	*Motacilla maderaspatensis*	Large Pied Wagtail	R
43.	Passeriformes	Passeridae	*Passer domestica*	House Sparrow	R
44.	Accipitriformes	Accipitridae	*Milvus migrans*	Black Kite	R
45.	Anseriformes	Anatidae	*Anas platyrhynchos*	Mallard	RM
46.	Passeriformes	Oriolidae	*Oriolus xanthornus*	Black Headed Oriole	R
47.	Passeriformes	Cettiidae	*Abroscopus superciliaris*	Yellow-bellied warbler	R

R = Resident ; RM = Resident Migrant; M = Migratory

Table 4: List Seasonal Variation of avifauna within Jalangi River, Nadia District, West Bengal

Common Name	Availability during Winter and Spring	Availability during Rest of the Year
Common Greensank	Yes	No
Wood Sandpiper	Yes	No
Common Sandpiper	Yes	Yes
Common Snipe	Yes	No
Marsh Sandpiper	Yes	No
Phesant tailed Jacana	Yes	Yes
Bronzed winged Jacana	Yes	Yes
Red-wattled Lapwing	Yes	Yes
Grey-headed Lapwing	Yes	No
Great Cormorant	Yes	Yes
Little Cormorant	Yes	Yes
Little Grabe	Yes	No
Grey Heron	Yes	No
Large Egret	Yes	Yes
Indian Pond Heron	Yes	Yes
Median Egret	Yes	Yes
Little Egret	Yes	Yes
Black-headed Ibis		RM
White-breasted Waterhen	Yes	Yes
Common Moorhen	Yes	Yes
Common Coot	Yes	Yes
Asian Openbill	Yes	Yes
Lesser Whistling Duck	Yes	Very few
Greater Coucal	Yes	Yes
Lesser Pied Kingfisher	Yes	Yes
Blue eared Kingfisher	Yes	Yes
White-breasted Kingfisher	Yes	Yes
Small Green Bee-eater	Yes	Yes
Lesser Golden Backed Woodpecker	Yes	Yes
Common Hoopae	Yes	Yes
Common Swallow	Yes	Yes
Brown Shrike	Yes	No
Burmese Shrike	Yes	No
Black Drongo	Yes	Yes
Asian Pied Starling	Yes	Yes
Bank Myna	Yes	Yes
House Crow	Yes	Yes
Jungle Crow	Yes	Yes
Plain Prinia	Yes	Yes
Oriental Magpie Robin	Yes	Yes
White Wagtail	Yes	No
Large Pied Wagtail	Yes	Yes
House Sparrow	Yes	Yes
Black Kite	Yes	Yes
Mallard	Yes	Yes
Black Headed Oriole	Yes	Yes
Yellow-bellied warbler	Yes	Yes

Fig. 1: Red Walted Lapwing

Fig. 2: Brown Shrike

Fig. 3: White pied king fisher

Fig. 4: Common Sandpiper

Fig. 5: Green Bee-eater

Fig. 6: Grey Heron with Small Egret

Fig. 7: Leaser Whistling Duck

Fig. 8: Black-headed Ibis

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Review Article

Modification of Aerial Microenvironment and Its Impact on Wheat (*Triticum Aetivum* L.) in Agroforestry Systems

Ravi Kiran

Abstract

The understory crop in an agroforestry system exoerience varied effect of trees besides the modified microenvironment. Under changing climatic scenarios and crunch of narural resources agroforestry is proved boon. The positive interactions at the tree-crop interface are reduction of heat load and proper nutrient balance. For wheat, ingeneral shading caused decrease in yield and yield components due to low photosynthetic leaf area. This paper discusses the Modification of aerial microenvironment in an agroforestry systems.

Keyworde: Wheat (*Triticum Aetivum* L.) ; Crop Growth; Modified Aerial Microenvironment; Agroforestry.

Introduction

Emission of greenhouse gases has now a days become a matter of great concern over the globe. The reduction in concentration of CO_2 in the atmosphere can be achieved by removal of the atmospheric CO_2 through carbon sequestration. agroforestry can increase the amount of carbon stored in lands for agriculture, while still allowing for growing of food crops .

Global circulation climate models predict an increase in mean ambient temperatures between 1.8 and 5.8^{0}C by the end of this century (IPCC, 2007).

Exposure to excessive temperatures during development reduces the yield of wheat. High global temperatures and frequent heat waves are likely to have similarly negative effects on natural systems. (yadav, 2010) Tree canopies can provide suitable microclimatic conditions for growth and development of wheat. Hot weather with dry wind is injurious to wheat plant at grain filling.

These decrease in photosynthetic rate and chlorophyll content in wheat plant. Grain filling is not greatly affected by short shading period but increasing the length of period of shading brought about an accelerating yield reduction.

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Wheat Performance under Modified Aerial Microenvironment

In poplar-wheat agroforestry system, growing condition of wheat are modified due to presence of tree and, thus, response of wheat differs than that of tree-less agricultural system. Age of poplar trees is recorded as most important factor influencing wheat grain (var. PBW 343) yield. On an average, reduction in grain yield was 20.10% under 1-year-old poplar plantation, which increased to 54% under 4-year-old plantation. Under irrigated poplar-based agro-ecosystem, light is the major limiting factor for reduction in grain yield (Chauhan *et. al*, 2009).

Denmead (1969) studied about comparative micrometeorology of a field of wheat and a forest of *Pinus radiata*. For a given short wave radiation intensity, quite large differences existed between communities in net radiation, evaporation,

photosynthesis and soil heat flux. The first three were greater for forest and last was smaller. Wheat had higher foliage temperature than air but opposite for forest.

Dhillon *et al.* (1979) grew eucalyptus trees in N - S and E - W direction to study the effect of trees on adjoining crops. Reduction in grain / paddy yields of wheat and rice grown on the S aspect of E - W line of trees was greater than when grown on the N aspect. In general, reduction in yields of crops grown along the E - W tree line was relatively less than when they were grown along N - S line.

Sheikh and Haq (1978) showed yield reduction in wheat in quadrats at distances of 2 - 9 in 8 directions from 5 trees of *Acacia arabica* and 5 of *Dalbergia sissoo*. The yield was significantly reduced by shading. The effect was found decreased with distance from the tree. Yield was lowest in the sample taken from the north side of trees.

Dhillon *et al.* (1982) showed that the yield of wheat planted with eucalyptus trees was less reduced in N - S direction plantation than E - W direction plantation of trees. Intercropped potato showed more tolerance to shading effects as compared to paddy and wheat crops.

Sheikh *et al.* (1983) reported no significant difference in any measurement of wheat intercropped with 4 year old hybrid poplars . The total grain yield was also not effect significantly below trees.

Makasharipova *et al.* (1985) studied the effect of shading either through increased stand density or by using screens on wheat and found that ingeneral shading caused decrease in yield and yield components due to low photosynthetic leaf area.

Cole and Newton (1986) studied about folair and soil nutrient, canopy light penetration and predawn moisture stress in Douglasfir on Nelder plots. Canopy light penetration varied with competitor, density and height, above ground, the lowest value occuring under red alder canopy. It was concluded that grass competed primarily for moisture and that red alder reduced available light and moisture.

Hazra and Patil (1986) found that the infiltrated light below *Albizia lebbeck, Acacia procera, Lucaena leucocephala* and *Acacia tortilis* varied from 74-93 per cent of PAR than that of open sites. High R.H. below tree canopies (62-70 per cent) was another feature than open field (56 per cent).

Basu *et al.* (1987) studied the allelopathic effect of *Eucalyptus terelicornis* on the potato and wheat. Potato yield was greatly reduced in the plots near the eucalyptus. Field observation of wheat growth indicated marked reduction in plots near the eucalyptus.

Green (1987) reported that in wheat, fertilizer nitrogen accelerated the rate of canopy expansion giving greater canopy size and improving fraction and quantity of radiation absorbed by the foliage. The rate of crop growth from stem elongation to near maturity was constant while the quantity of irrigation increased.

Balsky *et al.* (1989) reported the reduction of 45-65 per cent in solar radiation, in soil temperature 5-11^{o}C and in rain fall by 50 per cent under the tree conopies of *Acacia tortilis* and *Adansonia digitata.*

Cameron *et al.* (1989) conducted an experiment in which *Eucalyptus grandis* was planted in a Nelder fan design into a setaria dominated pasture and reported that pasture growth was little affected by trees at the tree age of 0.5 years, but substantially reduced after 1.5 years under more than 1000 stems per hectare. By age 3.5 years, pasture production were reduced significantly under the tree canopies.

Ong *et al.* (1991) investigated about the above and below ground interaction such as change in light, temperature, humidity and soil moisture and found the effect of these on understorey crops. Atmospheric interaction was found positive in alley cropping in semi-arid tropics but a minor importance compared with the below ground interactions. The competition for soil moisture between two components was reponsible for negative interactions in semi-arid tropics.

Messing and Noureddine (1991) studied the effect of wind break on wind velocity, potential evapotranspiration, temperature, crop growth and water use efficiency on wheat. Both artificial and biological wind breaks were used. Wind velocity behind the artificial wind breaks varied between 30 to 60 per cent depending on the distance from wind break. A 60 per cent reduction in wind velocity was found at 4H (H was the height of wind break) with Acacia and Casuarina. The presence of wind breaks also decreased potential evapotranspiration, increased wheat growth and increased water use efficiency.

Corlette *et al.* (1992) reported that alley cropping of millet and *Leucaena leucocephala* changed the microclimatic conditions. *L. leucocephala* reduced the wind speed and incident light substantially. Leaf and soil temperatures within the alleys found warmer during night and cooler during the day than pure millet.

Wang and Shogren (1992) showed that paulownia intercropping with winter wheat, resulted in more

efficient use of water and other limited resources and paulownia trees on crop land enhanced the microclimate and, therefore, increased the wheat yield and quality.

Pant (1993) measured the light available under tree canopy of different tree species and sole crop using a protable lux meter during morning (8.30-10.30), noon (11.30-1.30) and evening (3.00-4.30) hrs. at fifteen days intervals, right from July 1989 to June 1990. The reduction in average light availability, both under poplar and guthel during winters was only about 31 percent while during summer and rainy season it was as high as 56 percent. Light reduction was more pronounced during morning and evening hours as compared to noon hours under all the tree species.

Nazir *et al.* (1993) studied the effect on crop of wheat under *Dalbergia sissoo* and found that increasing duration of shading decreased plant height, number of fertile tillers/unit area, number of grains/spike, 1000-grain weight, grain protein concentration, percentage DM and grain yield.

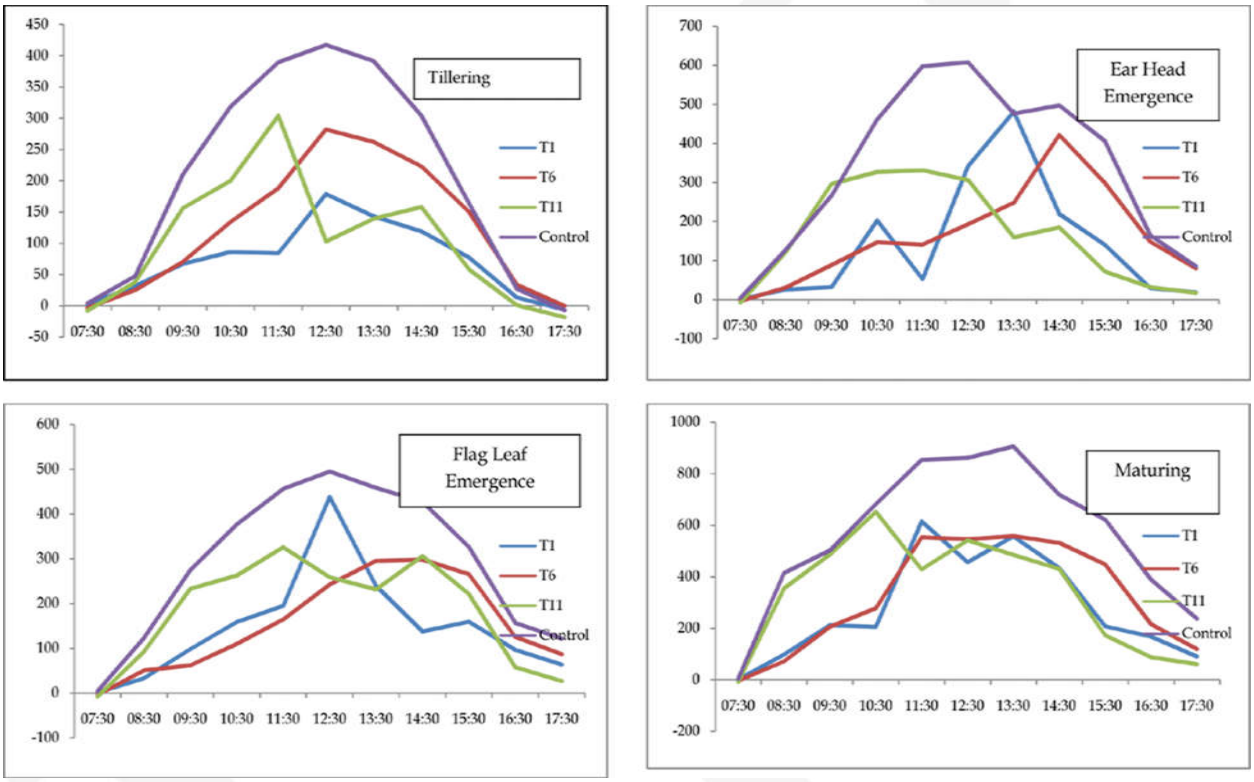

Fig. 1: Diurnal variation in net radiation (W/m²) at different stages of wheat intercropped with Eucalyptus in a Nelder Wheel Design from 07:30 to 17:30 Hrs
Source: Ravi Kiran, 1997

Jiang *et al.* (1994) analysed the paulownia intercropping types and their benefits and found that paulownia trees could improve the micorclimate by reducing wind speed (21-50 per cent) in May and by increasing relative humidity in summer which favoured the wheat yield.

The present field investigation was carried out at the Horticultural Research Center, Patharchatta, located in the campus of GB Pant University of Agriculture and Technology, Pantnagar, India (29° N, 79° 30 E, 243.83 m above mean sea level). *Eucalyptus tereticonis* was planted in a Nelder wheel design in March 1989 (Nelder, J.A. 1962). It consisted of fifteen tree rows each oriented at an angle of 24° from the adjacent tree row, having ten trees in each row at a distance of 2.0, 5.4, 13.5, 17.8, 21.5, 24.6, 27.4, 29.9, 32.2 and 34.4 m respectively, enclosing a total number of 15 plots between the tree rows. The plots were serially numbered from 1 to 15 anticlockwise starting from a tree row oriented to 0° in north direction and divided into three sub plots of area 26.62 m² each for the propose of the investigation. The first, second and the tenth trees were considered as buffer trees to avoid border effect. Therefore, a constant tree stand of 333 trees per hectare was provided in the area between the third and the ninth trees of each row resulting each tree in the experimental area occupying an average area of 30 m². Tree rows were given the treatment of pruning of 33% of tree height for proper radiation penetration below tree canopies. Diurnal changes in Net radiation (Rn) over wheat crop in the treatment 1, 6 and 11 and in control was recorded

from 0730 to 1700 h. The data show that wheat crop received 37, 60 and 50% Rn of the control in treatment 1, 6 and 11, respectively and the maximum values of Rn were 234, 341, 314 and 426 W/m^2 in treatment 1, 6, 11 and control, respectively at tillering stage. Diurnal changes in Rn over the wheat crop in treatment 1, 6, 11 and in control show that wheat crop received 43, 49 and 50% Rn of the control, respectively and the maximum values of Rn were 546, 436, 475 and 663 W/m^2 in treatment 1, 6, 11 and control, respectively at Flag leaf emergence stage. Diurnal changes in Rn over wheat in treatment 1, 6 and 11 show that wheat crop received 50, 52 and 64% Rn of control during flowering stage of wheat crop and the maximum values of Rn were 509, 350, 380 and 504 W/m^2 in treatment 1, 6, 11 and control, respectively at Flowering stage. Diurnal changes in Rn over wheat in treatment 1, 6 and 11 show that crop received 48, 58 and 62% Rn of the control and the maximum values of Rn were 573, 622, 735 and 923 W/m^2 in treatment 1, 6, 11 and control, respectively at Maturing stage (Ravi Kiran, 1997) (Figure 1).

Jiang *et al.* (1994) showed that the effect of crown shading on photosynthetically active radiation (PAR) was not significant on the number of effective spikes and grain of wheat, but it affected total grain yield and 1000 grain weight, with size of the effect depending on the distances from the trees.

A study was conducted to explore the carbon sequestration potential of agroforestry systems specifically poplar-wheat-based system. Total CO2 assimilation by the biomass in the poplar-wheat-based agroforestry system and monocropping of poplar and wheat was estimated at 28.6, 17.2 and 17.8 t/ha/year, respectively. It is established that agroforestry in irrigated agroecosystems, such as the poplar-wheat integrated cropping system, store more carbon in above- and below-ground biomass than sole crop cultivation. (Chauhan and Chauhan, 2009)

Conclusion

In agroforestry excess shade may reduce the wheat yield mostly ; however, flactuating light may be beneficial for crop. In this way, agroforestry system can be used for changing the microclimatic conditions for the intercrops for beneficial purpose.

There is a decreased potential evapo-transpiration, increased wheat growth and increased water use efficiency in agroforestry. Trees intercropping with winter wheat, resulted in more efficient use of water and other limited resources and paulownia trees on crop land enhanced the microclimate and, therefore, increased the wheat yield and quality. Further research is needed on the exploitation of this aspect.

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Revised Rates for 2017 (Institutional)

Title	Frequency	Rate (Rs): India		Rate ($):ROW	
Community and Public Health Nursing	3	5000	4500	357	300
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Indian Journal of Anatomy	4	8000	7500	571	500
Indian Journal of Ancient Medicine and Yoga	4	7500	7000	536	500
Indian Journal of Anesthesia and Analgesia	4	7000	6500	500	450
Indian Journal of Biology	2	5000	4500	357	300
Indian Journal of Cancer Education and Research	2	8500	8000	607	550
Indian Journal of Communicable Diseases	2	8000	7500	571	500
Indian Journal of Dental Education	4	5000	4500	357	300
Indian Journal of Emergency Medicine	2	12000	11500	857	800
Indian Journal of Forensic Medicine and Pathology	4	15500	15000	1107	1050
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Indian Journal of Hospital Administration	2	6500	6000	464	429
Indian Journal of Hospital Infection	2	12000	9000	857	800
Indian Journal of Law and Human Behavior	2	5500	5000	393	350
Indian Journal of Library and Information Science	3	9000	8500	643	600
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	9000	8500	643	600
Indian Journal of Medical & Health Sciences	2	6500	6000	464	410
Indian Journal of Obstetrics and Gynecology	4	9000	8500	643	600
Indian Journal of Pathology: Research and Practice	4	11500	11000	821	780
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Indian Journal of Research in Anthropology	2	12000	11500	857	800
Indian Journal of Surgical Nursing	3	5000	4500	357	300
Indian Journal of Trauma & Emergency Pediatrics	4	9000	8500	643	600
Indian Journal of Waste Management	2	9000	8000	643	579
International Journal of Food, Nutrition & Dietetics	3	5000	4500	357	300
International Journal of Neurology and Neurosurgery	2	10000	9500	714	660
International Journal of Pediatric Nursing	3	5000	4500	357	300
International Journal of Political Science	2	5500	5000	550	500
International Journal of Practical Nursing	3	5000	4500	357	300
International Physiology	2	7000	6500	500	450
Journal of Animal Feed Science and Technology	2	78000	70000	5571	5000
Journal of Cardiovascular Medicine and Surgery	2	9500	9000	679	630
Journal of Forensic Chemistry and Toxicology	2	9000	8500	643	600
Journal of Geriatric Nursing	2	5000	4500	357	300
Journal of Medical Images and Case Reports	2	5000	4500	357	300
Journal of Microbiology and Related Research	2	8000	7500	571	520
Journal of Nurse Midwifery and Maternal Health	3	5000	4500	357	300
Journal of Organ Transplantation	2	25900	25000	1850	1700
Journal of Orthopaedic Education	2	5000	4500	357	300
Journal of Pharmaceutical and Medicinal Chemistry	2	16000	15500	1143	1100
Journal of Practical Biochemistry and Biophysics	2	5500	5000	393	340
Journal of Social Welfare and Management	3	5000	4500	357	300
New Indian Journal of Surgery	4	7500	7000	536	480
New Journal of Psychiatric Nursing	3	5000	4500	357	300
Ophthalmology and Allied Sciences	2	5500	5000	393	340
Otolaryngology International	2	5000	4500	357	300
Pediatric Education and Research	3	7000	6500	500	450
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Review Article

"Miracle Tree" *Moringa oleifera* Its Nutritive Importance and Safety Efficacy

Sanchita Pal*, Tanmoy Ghosh, IndraniI Bhattacharjee*****

Abstract

Moringa oleifera leaves, seeds, bark, roots, sap, and flowers are widely used in more than 80 countries to relieve mineral and vitamin deficiencies. It supports a healthy cardiovascular system, promote normal blood-glucose levels, neutralize free radicals {thereby reducing malignancy}, provide excellent support of the body's anti-inflammatory mechanisms, enrich anemic blood and support immune system. It also improves eyesight, mental alertness and bone strength. It is one of the richest plant sources of Vitamins A, B, C, D, E and K. The vital minerals present in *Moringa* include Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. It has more than 40 natural anti-oxidants. *Moringa* is an edible extremely safe plant. It can be easily and cheaply cultivated. Besides, *Moringa* has a direct impact on agriculture, water, sanitation, biodiversity and environment. *Moringa* responds to environmental and financial sustainability, realism and results, innovation, biodiversity, education and awareness. A wide variety of polyphenols and phenolic acids as well as flavonoids, glucosinolates, and possibly alkaloids is extracted from its different parts and believed to be responsible for biological activities including antioxidant, tissue protective (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, radio protective, and immuno modulatory actions. Standardization of products is an issue before its wide application in human beings.

Keywords: *Moringa Oleifera*; Parts; Benefits; Nutritional Value; Therapeutic Use; Safety Case.

Introduction

Mineral element deficiencies and Protein-energy malnutrition affects mostly children in poor countries (de Onis et al., 1993; Worldwatch Institute, 2011; Gonzalez 2015), with plant foods being key tools in addressing this situation. In 2011 alone, some 45% of all child deaths involved under nutrition (Black et al., 2013). Global priority is therefore to improve access to healthy food (de Onis et al., 1993). Most of the world's poor live in the tropics and of these the majority live in seasonally dry lowlands (Black et al., 2008, 2013; Sachs 2001; Food and Agriculture Organization of the United Nations, 2015). The development of plants not only with high nutrient levels but also exceptional drought resistance is essential. "Life on this planet has been likened to a pyramid with an unbelievably wide base and a small apex. Humans are somewhere near the top but not at the top because they are omnivores. They are one of those animals that can subsist on a wide range of food: vegetable and animal" (Seymour 2003). Plants have always been vital for mankind irrespective of the era and area all over the globe since the beginning of life. Edible wild indigenous plants become an alternative source for food possessing high potential of vitamins, minerals and other interesting elements particularly during seasonal food storage. Wild fruits are also known to have nutritional and medicinal properties that could be attributed to their antioxidant

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effects as well as being used to fortify stable foods particularly for malnourished children (Compaore et al., 2011).

Moringa oleifera Lam. tree grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia. It is well known vegetable in Africa, Arabia, India, Southeast Asia, America and Pakistan (Sengupta and Gupta, 1970). The genus *Moringa* have thirteen species in the world and it is a monogeneric family belonging to the Moringaceae and having two species in India viz. *M. concanensis* Nimmo ex Dalz. and Gibs. and *M. oleifera* Lam. Its roots, fruits, leaves and flowers been used as vegetables (Siddhuraju and Becker, 2003). The leaves are potential source of vitamin A and C, iron, calcium, riboflavin, β-carotene and phenolic acid (Nambiar et al., 2005). Its leaves and oil are a powerful natural antioxidant (Njoku and Adikwu, 1997). Siddhuraju and Becker (2003) observed antioxidant properties in the solvent extract of moringa leaves. Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. Leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids (Moyo et al., 2011; Teixeira et al., 2014; Razis et al., 2014). Additionally, leaves reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani et al., 2013; Vongsak et al., 2014). According to several authors (Anwar et al., 2007; Mbikay, 2012; Razis et al., 2014), various preparations of *M. oleifera* are used for their antiinflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectant activities. The therapeutic potential of *M. oleifera* leaves in treating hyperglycemia and dyslipidemia was reviewed by Mbikay (2012). Razis et al., (2014) summarized potential health benefits of *M. oleifera*, focusing on their nutritional content as well as antioxidant and antimicrobial characteristics.

Objectives of the Study

The concept of biodiversity in the form of food based supplementation as a mechanism to possibly provide diversity to the diets of people is prominent in nutrition research on food sources available. It is a concern that the original or cultural knowledge of people concerning foods and the nutritional value thereof are being lost to diet transition with migration and urbanization (Kuhnlein, 2003). The food compositions of a diverse range of foods have been analyzed and more still have to be analyzed as initiated in FAO projects lately (FAO, 2008). Johns (2003) and Kuhnlein (2003) suggests that with the knowledge of nutritional analysis, a range of foods could be introduced to supplement the diet of a household to improve food and nutrition security. Both authors view more research on traditional foods, such as traditional leafy vegetables, and its contribution in micronutrient contents as a positive step towards health promotion or intervention strategies.

The review aims to investigate the usage of *M. oleifera* leaves in the diets of existing users and to investigate the possibility of introducing the plant to households in need of diversifying their dietary intake. Considering all the above facts the present study is being undertaken to throw light on the different aspects of this plant in this overview.

Identifying Characters of Different Parts of M. oleifera

Moringa is a fast growing tree which can reach 12 m in height at maturity. The stem of the *Moringa* tree is normally straight, but occasionally poorly formed. The tree grows with short straight stems and can reach a height of 1.5 to 2 m before it begins branching out (Rajangam et al., 2001). The branches usually grow in a disorganized manner and the canopy is umbrella-shaped. The leaves are of a compound leaf form, with three leaflets arranged on either side of the stem in pairs opposite each other, growing mostly at the branch tips. The leaves are 20 to 70 cm long with 8 to 10 pairs of pinnae, each bearing two pairs of opposite elliptic or obovate leaflets (Rajangam et al., 2001). The fruit is a green three lobed pod that hangs down from the branches and can be 20 to 60 cm in length. When dry, it opens into 3 parts. Each pod contains between 12 and 35 seeds (Rajangam et al., 2001). The seeds are round, with brownish semi–permeable seed hulls. The hull itself has three wings that run from the top to the bottom at 120 degree intervals. The average weight per seed is 0.3 g. *Moringa* can be cultivated from cuttings and from seeds.

Nutritive Value of M. oleifera

The leaves of the tree contain balanced levels of essential amino acids as well as high levels of protein, calcium, and vitamin A (Sena et al., 1998; Makkar and Becker, 1996; Freiberger et al., 1998). Consequently, the plant is used extensively for low-cost nutrition (Thurber and Fahey 2009; Zongo et al., 1991; Fahey 2005). All parts of the tree are used medicinally and appear to have potent antioxidant, cancer chemo preventive, and gluco regulatory

activity (Fahey 2005; Bennett et al., 2003; Tumer et al., 2015; Siddhuraju and Becker, 2003). The seeds yield high-oleic oil used in cooking, cosmetics, and as a machinery lubricant (Tsaknis et al., 1999; Anwar and Bhanger, 2003). After oil extraction, the remaining seed cake can be used to clarify turbid water or to increase protein in animal feed or crop fertilizer (Folkard and Sutherland, 2002; Sarwatt et al., 2002; Baptista et al., 2015). Other uses include leaf extract as a leaf-applied fertilizer (Foidl et al., 2001). Despite the clear utility of the tree, crucial information gaps impede its optimal use in all of these applications, including nutrition.

According to Fuglie (2001), *Moringa* has gained popularity as a source of nutrition that can feed the needy and save lives as well. *Moringa* leaves or leaf powder can be used successfully as a complex food to nourish small children, pregnant women and nursing mothers as a treatment for malnutrition. The abundance of vitamin A in *Moringa* can contribute to the treatment of xerophthalmia (night blindness).

Fuglie (2001) and Marcu (2005) reported that *Moringa* leaves have about 40% protein with all of the nine essential amino acids present in various amounts. Because of this, *Moringa* is considered to have the highest protein ratio of any plant studied so far. It was reported that 100 g of *Moringa* leaves contain more than 200 mg of vitamin C and a high content of vitamin A in the form of provitamin A or β-carotene (Fuglie, 2001; Marcu 2005).

Leaves and pods of *Moringa* are rich in minerals and vitamins and could potentially be used in nutritional intervention programmes as a preventive measure against malnutrition. It has been observed that the nutrient composition of traditional vegetables has been recorded using different values, and furthermore unconfirmed data has been recycled in scientific and popular publications (McBurney et al., 2004). However, the high nutritional value of *Moringa* is widely recognized. Its value as a source of vitamin A is reported by Fuglie (2001).

Ramachandran et al., (1980) reported the vitamin A content of *Moringa* as 11,300 IU per 100 g edible portion. The original source did quote the value as β-carotene, which should read 11,300 IU β-carotene per 100g edible portion (McBurney et al., 2004). Babu (2000) reported vitamin A content as 3767 IU per 100 g edible portion. A publication of Kuhnlein (2003) quoted *Moringa* in Niger as containing 5880 µg β-carotene per 100 g edible portion. This data of Kuhnlein (2003) is recommended by McBurney et al., (2004). An initiative was launched by FAO to analyze the nutrient composition of traditional leafy vegetables so as to standardize the nutrient content per 100 g edible portion (FAO, 2008).

Fuglie (2001) recommends that 25 g of *Moringa* leaf powder equals one rounded tablespoonful of *Moringa* leaf powder which when added to infants' food three times per day would provide roughly the RDA with calcium and vitamin A 1.5 mg or 1500 µg exceeding the RDA by 310%. The vitamin A intake for the group children 1-3 years in South Africa was less than one out of two 55-66% that was half the recommended level (Labadarios, 2000). The vitamin A intake for children living rural in the age groups between 1-9 years was 62-73%. At national level less than one of two 55-68% children had a vitamin A intake that was half the recommended level (Labadarios, 2000).

The leaves are the most nutritious part of the plant, being a significant source of vitamin B6, vitamin C, pro-vitamin A as β-carotene, magnesium and protein, among other nutrients (Peter 2008). When compared with common foods particularly high in certain nutrients per 100 g fresh weight, *Moringa* leaves are considerable sources of these same nutrients (Makkar and Becker, 1997; Fuglie, 2001). Scientific research confirms that leaves of this plant are of significant nutritional value. Gram for gram, *Moringa* leaves contain: seven times the vitamin C in oranges, four times the Calcium in milk, four times the vitamin A in carrots, two times the protein in milk and three times the Potassium in bananas (Hsu et al., 2006). It also contains Vitamins B1, B2, B3, B6, B7, D, E, K, and amino acids isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparatic acid, cysteine etc. Some of the calcium in *Moringa* leaves is bound as crystals of calcium oxalate which may inhibit calcium availability to the body (Olson and Carlquist, 2001).

Nutritional Benefits of Different Parts of M. oleifera

Leaves

Benefits

Moringa leaves are very rich source of vitamin A, C, Ca, K, protein and essential elements in comparison to locally available in market viz. Carrot, orange, cow milk, banana etc. The leaves may be supplemented as essential food. The leaves can be served to check malnutrition in the poor's. It is a nutraceutical and universal remedy for various diseases having 35 elements. Leaf powder can be used as hand washing product-hand hygiene to reduce gastrointestinal and respiratory illness. Tender twigs and immature pods used as fodder for cattle's to increase milk. Pregnant woman consumed leaves and flowers to increase milk

for infants. Leaf powder used as biocontrol in crops, as fertilizers and pesticides.

Phytochemistry

Leaves contain high iron, minerals, vitamins and proteins. It also contains 14 macroelements and 21 microelements (total 35 elements). During hand washing the mechanical friction by the dry leaf powder reduces the bacterial effect in comparison to non-medicated liquid soap (Asiedu-Gyekye et al., 2014; Anwar et al., 2007; Fozia et al., 2012; Kamal 2008; Khawaja et al., 2010; Paliwal 2011b; Paliwal 2011a; Parrotta et al., 2009; Wealth of India, 2001).

Stem

Benefits

Stem pulp used to make newspaper and textile industries. Corky bark yield fibers used in making mats, paper, cordages etc.

Phytochemistry

Presence of Cellophane (Parrotta et al., 2009; Wealth of India, 2001)

Pods

Benefits

Immature pods cooked as vegetable or pickled, having high nutritional and medicinal value.

Phytochemistry

Contains higher percentage of vitamins essential elements, glycosides etc (Parrotta et al., 2009; Wealth of India, 2001).

Seeds

Benefits

Seed powder paste used as water purifier to improve the quality of drinking water by absorbing the heavy metals viz. Cadmium, Copper, Chromium, Lead and Zinc which are highly toxic to human being. The seeds can be used as nutritional supplements for industrial and agriculture purpose. It is also being used in perfume industries, cosmetic, lubricate, soap as antioxidant activity oil used as body cream. It can also used as vegetable in daily consumption.

Phytochemistry

Moringa has cationic polyelectrolyte of short chain and low, molecular weight. Heavy metals having higher charges are trapped by seed powder. Seeds oil locally known as "*ben oil*" "*Drumsticks*" similar to olive oil and is rich in Palmetic, stearic and oleic acids. The oil is clear, odourless and resists rancidity, oil possesses 75% oleic acid and therefore used in perfume and soap industry (Ojiako and Okeke, 2013).

Chemical Composition of Different Parts of Moringa oleifera

The chemical composition of different parts of *Moringa* by Massry et al., 2013 reveals that that seeds of *M. oleifera*, as other legumes, are good sources for proteins and crude fibers. It contained 44.78, 25.97 and 4.87 % (on dry weight basis), respectively. Also, dried leaves contained high amounts of protein and crude fibers which were 26.79 and 18.67 %, respectively. Total carbohydrates contents were higher in fresh and dried leaves of *M. oleifera*, which were 37.85 and 35.90%, respectively. In addition the ash content was 3.64 and 7.92%, respectively

Mineral Contents of Different Parts of M. oleifera

M. oleifera contains different minerals. It contains high concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K) and sodium (Na) in dried leaves than those of *Moringa* other parts. The concentrations of the aforementioned minerals were 2078.98, 346.87, 403.56, 1498.75 and 72.50 mg / 100 g (DWB) respectively. The seeds contain also the same minerals contents, which were nearly similar to those in *M. oleifera* leaves, which were, 76.85, 524.30, 259.78, 64.24 and 24.92 mg / 100 g (DWB). On the other hand, *Moringa* seeds contained appreciable amounts of minerals especially microelements such as; zinc (Zn), copper (Cu) and manganese (Mn). The values obtained for the microelements, Zn (27.47), Cu (48.13) and Mn (87.75) mg / 100 g (DWB), respectively (Massry et al., 2013).

Amino Acids of Different Parts of M. oleifera

M. oleifera contained 18 (eighteen) amino acids. Argenine, glutamic acid and cystine in *M. oleifera* seeds protein were the most predominant amino acids which contents are 12.68, 18.76 and 4.59 g/ 16 g N, respectively. On the other hand, *M. oleifera* leaves contained high amount of other amino acids, especially essential amino acids such as, methionine, valine, phenylalanine, leucine, lysine and tryptophan, which were 2.12, 6.47, 6.38, 10.12, 6.73 and 2.17 g / 16 g N, respectively (Massry et al., 2013).

Natural Antioxidants and Antioxidant Activities of M. oleifera Different Parts

Total polyphenols, total flavonoids, ascorbic acid, â-carotene, carotenoids and total antioxidant activity are found in different parts of *M. oleifera*. Total polyphenols, total flavonoids and ascorbic acid of different *Moringa* plant parts ranged from (9.57 to 22.38), (68.97 to 142.20) and (67.84 to 871.28) mg / 100 g (DWB), respectively. On the other hand, β-carotene and carotenoids ranged from (0.65 to 28.36) and (28.94 to 149.95) mg / 100 g (DWB), respectively in different *Moringa* plant parts. Total antioxidant activity also ranged from (133.78 to 168.34)%, for pods and leaves (Massry et al., 2013).

Identification of Phenolic Compounds of Different Parts of M. oleifera by HPLC Analysis

Ten phenolic compounds were identified from *Moringa* different parts by High Performance Liquid Chromatography (HPLC) analysis. The detected phenolic compounds were gallic acid, chlorogenic acid, ellagic acid, ferulic acid, kaempferol, quercetin , rutin, syringic acid, caffeic acid and catechin. The highest contents of phenolic compounds were quercetin, kaempferol and rutin for pods, seeds and fresh and dried leaves, respectively. The above mentioned values were 42.36, 74.13 and 97.68 mg/ 100g (DWB), respectively. quercetin ,caffeic acid and kaempferol were predominant phenolic compounds in *Moringa* pods and seeds extracts. Whereas, the rutin, caffeic acid and ferulic acid are the dominant phenolic constituents of *Moringa* leaves extracts (Massry et al., 2013).

Therapeutic Uses of Different Parts of M. oleifera

Phytochemicals refers to only those chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients. *Moringa* contains a range of fairly unique phytochemicals containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. Six phytochemicals have been reported to have hypotensive, anticancer, and antibacterial activity include benzyl isothiocyanate , niazimicin , pterygospermin**,** benzyl isothiocyanate**,** and 4-{a-L-rhamnopyranosyloxy} benzyl glucosinolate (Costa-Lotufo et al., 2005; Fahey et al., 2004; Faizi et al., 1998; Fuglie 1999; Fuglie 2000; Fuglie 2001).

Numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with *Moringa* or with phytochemicals isolated from *Moringa* have shown, antiulcer, effect on immune response, spasmolytic activities, hypocholesterolemic effects, antibacterial activity. sympatholytic activity and antiviral activity against herpes simplex virus type-1 (Galan et al., 2004; Ghasi et al., 2000; Gilani et al., 1994; Hameed-Un-Nisa et al, 1998; Haristoy et al., 2005). Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. The data obtained suggests that the extracts of *M. oleifera* both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage (Sreelatha and Padma, 2009; Yongbai 2005).

Roots

Therapeutic uses

The roots aqueous extract and dry root powder is antilithic, rubefacient, vesicant, antispasmodic, hepatoprotective, carminative, antifertility, antiinflammatory, stimulant in paralytic condition; considered as cardiac/circulatory stimulant, laxative, abortifacient, rheumatism, joints pains, lower back pains or in renal pains. The juice of root- bark is said to very effective if put in ear and tooth cavity as a pain killer, and has antitubercular activity.

Phytochemistry

The aqueous and ethanol extract of roots having o-sito-sterol, alkaloid moringinine which act as cardiac stimulant. (Caceres et al., 1992; Dahot 1988; Duke 2001; Khare et al., 1997; Padmarao et al., 1996; Ruckmani et al., 1998).

Stem-Bark

Therapeutic Uses

The aqueous stem extract used to cure as rubefacient, vesicant, eye infections, prevent enlargement of spleen and formation of tuberculosis glands of the neck, to destroy tumours and to heal ulcers, antibacterial activity.

Phytochemistry

Contain two alkaloids namely moringine and moringinine, vanillin, â-sitosterol, â-sitostenone, 4-hydro-xymellin and octacosanoic acid have been

isolated from the stem-bark (Bhatnagar et al., 1961; Faizi, 1994a; Faizi 1994b; Ghasi et al., 2000; Kerharo 1969; Siddhuraju and Becker, 2003).

Leaves

Therapeutic uses

Leaves extracts used in malnutrition to supplement vitamins, essential elements, proteins etc., antihypertensive, diuretic, cholesterol lowering, blood pressure control, antipyretic, decrease blood glucose concentration, antidiabetic, antioxidant, hepato-protective, reduces liver fibrosis, anticancer-antitumor activities, antifertility, antispasmodic, antiulcer, gastrointestinal disorder, cardiac and circulatory stimulant, eye or ocular diseases like night blindness and ear infection, bronchitis, antiasthma, analgestic, antimicrobial, antibacterial, antifungal etc. Leaves juice applied externally to cure scurvy, sores, temples for headache, piles, sores of throat, glandular swelling, eye and ear infection, etc.

Phytochemistry

Antibacterial effect is shown due to presence of Pterygospermin. Antifungicidal effects is shown by 4 - (4'-o-acetyl – a – L - rhamnopyranosy - loxy) benzyl isothiocyanate, 4 (a – L - rhamnopyranosyloxy) benzyl isothiocyanate, nizimicin, isothiocyanate and 4 (a-L-rhamnopyranosyloxy). It also contains alkaloid Moringine-as antiasthmatic. Nitrile, mustard oil glycosides and thiocarbamate glycosides helps in lowering blood pressure. Dark chocolate polyphenols and other polyphenols have hypoglycaemic or antidiabetic effect. Quecetin and kaempferol used as antioxidant and hepatoprotective. Niazimicin has anticancer properties. The leaves act as a good source of natural antioxidant due to presence of various types of antioxidant compounds such as ascorbic acid, flavonoides, phenolics and carotenoids, other major and minor essential elements, vitamins, amino acids (Agrawal and Mehta, 2008; Al-Awwadi et al., 2004; Anwar et al., 2005; Bajpai et al., 2005; Bose 1980; Dahot, 1988; Fahey 2005; Faizi et al., 1995; Faizi et al., 1994a; Faizi et al., 1994b; Faizi et al.,1998; Anwar et al., 2007; Fozia et al., 2012; Fuglie 2001; Ghada 2013; Gilani et al., 1994; Grassi et al., 2005; Guevaraa et al., 1999; Kirtikar and Basu, 1975; Moharram et al., 2003; Makkar and Becker, 1996; Makonnean et al., 1997; Morton 1991; Nwosu and Okafor, 1995; Ojiako and Okeke, 2013; Paliwal 2011b; Paliwal 2011a; Rao et al., 1946; Ruckmani et al., 1998; Selvkumar and Natarajan 2008; Siddhuraju and Becker, 2003; Sikder et al., 2013; Trees For Life, 2005 ; Wealth of India, 2001).

Flowers

Therapeutic uses

Highly medicinal value as a stimulant, aphrodisiac, abortifacient, cholagogue, used to cure inflammations, muscle diseases, hysteria, tumours and enlargements of the spleen, useful in lowering the serum cholesterol, phospholipids, triglyceride, VLDL, LDL cholesterol to phospholipids ratio atherogenic index; decrease lipid profile of liver, heart and aorta.

Phytochemistry

Flowers contain nine amino acids, Sucrose, D-glucose, traces of alkaloids, wax, quercitin and kaempferat; the ash is rich in potassium and calcium. It also reported to contain some flavonoid pigments such as alkaloids, kaempherol rhamnetin, isoquercirin and Kaempleritrin (Bhatnagar et al., 1961; Ruckmani et al., 1998; Siddhuraju and Becker, 2003).

Seeds/pods

Therapeutic uses

The seed extract if taken orally very effective in decreasing liver lipid peroxides, antihypertensive. The seeds are antipyretic, acrid, bitter and antimicrobial activity. The seed can be consumed fresh as peas or pounded, roasted, or pressed in to sweet, non-desiccating oil, commercially known as '*Ben oil*' of high quality. ethanol and aqueous extracts of whole pods, seed-coat, pod pulp revealed that the B.P. lowering effect of seed is more pronounced.

Phytochemistry

The antihypertensive compounds thiocarbamate and isothiocyanate glycosides have been isolated from the acetate phase of the ethanolic extract of the pods. The unique property is the ability of its dry, crushed seed and seed press cake, which contain polypeptides, to serve as natural coagulants for water treatment as purifier. The seed oil contains sterols. The sterol composition of the major fractions of *M. oleifera* seed oil differs greatly from conventional vegetable oils. The seed oil is having high oleic acid (Anwar and Bhanger, 2003; Anwar et al., 2005; Faizi et al., 1998; Guevaraa et al., 1999; Ndabigengesere and Narasiah, 1998; Nagar et al., 1982; Oliveira et al., 1999).

Pharmacological Effects of M. oleifera

Radio-Protective and Immunomodulatory Effect

Methanolic extract of *M. oleifera* leaves exerted a

radioprotective effect on radiation-induced chromosomal aberrations and micronuclei (Rao et al., 2001). Methanolic extract of leaves of *M. oleifera* was found to be more significant than other extracts during the study of immunomodulation due to the presence of flavonoids, polyphenols and terpenoids which may modulate the body's immune-mechanisms. Methanol extract stimulates both cellular and humoral immune systems, hence it plays a plausible role on the body's immunity (Bello and Nzeh 2013; Gaikwad et al., 2011).

Ameliorative Effect

M. oleifera leaf extract had a protective effect against the induced testicular toxicity induced by administration of chromium which evidenced by improvement in sperm parameters of experimental rats (Akunna et al., 2012).

Anti-Malarial Effect

Ethanolic extract of *M. oleifera* was found to have anti-malarial effect on some malaria-induced mice. This was also observed in Cyclophosphamide induced toxicity in mice (Gupta et al., 2009).

Anti-Microbial Effect

The powder from fresh leaf juice (dissolved in DMSO) has greater antibacterial activity than fresh leaf juice. Ethanol and water extracts of fresh leaf juice and ethanol extract of fresh leaves showed higher antibacterial potential than the corresponding water extracts when administered to about ten cultured pathogenic bacteria (Rahman et al., 2009).

Hypoglycemic Effect

Administration of *M. oleifera* extracts (root bark, stem bark and leaves) with hypoglycaemic properties was also observed to have lowered the blood sugar levels and could be used for the management of diabetes (Umar et al., 2007).

Other Effects/ Uses

Animal Feed Fortification

Moringa leaves when added to cattle feed increased their daily weight gain by up to 32 percent. Feed of milk cows was supplemented with 15 to 17 kilograms of fresh *Moringa* leaves daily, and the cattle's milk production increased by 43 percent. Feed supplemented with 2 kg dry matter and milk production increased by 58 percent (Foidl et al., 2001; Francis et al., 1991).

Plant Growth Enhancer

Lab experimentation had shown that *Moringa* spray had a wide range of beneficial effects on plant crops. Effects of *Moringa* spray indicated accelerated growth of young plants. Plants were firmer, more resistant to pests and disease. longer life-span, heavier roots, stems and leaves, produced more fruit, larger fruit, increase in yield 20-35% If even a fraction of these results could be reproduced in the field, it could be a great help in increasing food supplies for millions of hungry people (Foidl et al., 2001).

Water Purification

A billion of people across Asia, Africa, and Latin America are estimated to rely on untreated surface water sources for their daily water needs. Of these, some two million are thought to die from diseases caught from contaminated water every year, with the majority of these deaths occurring among children under five years of age. Powdered seed act as a natural flocculent, able to clarify even the most turbid water Seed powder can be used as a quick and simple method for cleaning dirty water. The powder joins with the solids in the water and sinks to the bottom. This treatment also removes 90-99% of bacteria contained in water, water purification by flocculation, sedimentation, antibiosis and even reduction of Schistosome cercariae titer. Using *Moringa* to purify water replaces chemicals such as aluminum sulphate, which are dangerous to people and the environment, and are expensive. Twenty litres of water may be purified by adding 2 grams of powder to one cup of clean water, pour into a bottle and shake for 5 minutes (Gassenschmidt et al., 1995; Jahn et al., 1986; Kumar and Gopal, 1999; Sutherland et al., 1989; Yongbai 2005).

Moringa Oil

The Romans, Greeks and Egyptians extracted edible oil from the seeds and used it for perfume and skin lotion. The extracted *M. oleifera* seed oil revealed an iodine value of 68.63; refractive index (40°C), 1.4571; density (24°C), 0.9032 g cm^{-3}; saponification value, 181.4; unsaponifiable matter, 0.74%; acidity (as oleic acid) 0.81% and color. The major sterol components of the oil were β-sitosterol (46.16%), campesterol (17.59%), stigmasterol (18.80%) and avenasterol (9.26%). The wild *M. oleifera* seed oil was found to contain oleic acid up to 73.22%, followed by

palmitic, stearic, behenic and arachidic acids 6.45, 5.50, 6.16 and 4.08%, respectively and fell in the category of high-oleic oils (Anwar and Bhanger 2003; Dahot and Memon, 1987; Farooq and Rashid, 2007; Fuglie 2001).

Recommendation and Future Prospects

The 21st century is the century of biology powered and derived by scientific knowledge and technology expertise. Three technologies viz. "*Biotechnology*", *Herbal technology*" and "*Information technology (Bioinformatics)*", all these technology are crucial for prosperity and welfare for the people of nations. All technologies for manufacture of value added plant products can be called as "*Herbal technology*".

The climate of India is favorable for the cultivation and plantation of this tree. The State forest Department should take initiative for this purpose. People should be educate and make them aware regarding multifarious use of this "*miracle tree*". India can easily fight against the problems of malnutrition, hunger, poverty, diseases, unemployment, and edible oil export by utilizing its full benefits.

The lot of foreign exchange could be earned by exporting the products of "*Moringa*" instead of spending foreign exchange on imports. This tree truly appears to be a Miracle plant having countless benefits for humanity and thus should be taken as a high quality gift of nature at very low price. The maximum cultivation of this plant in open wastelands, fallow fields, roadsides, around field boundaries, and around houses provides maximum yield of its different useable parts could be achieved to propel the maximal amount of commodities of a multifarious nature for the welfare of humankind.

In view of the edible nature of the plant, more research work can be done on human so that a drug with multifarious effects will be available in the future market. So the State and Central Government must take immediate initiative to plant widely this "miracle indigenous" tree in most of the areas where climatic condition are favorable for maximum yield of its different usable parts.

M. oleifera Lam. is an important source of naturally occurring phytochemicals and this provides a basis for future viable developments like health, socioeconomic developments, cosmetics, as water purifier, etc. Different parts of this miracle tree should also incorporate in various marketed health formulations, such as: Orthoherb (Water Bushnell Ltd., Mumbai, India), Rumalaya and Septilin (The Himalaya Drug Company, Bangalore, India), Livospin (Herbals APS Pvt. Ltd., Patna, India) and Kupid Fort (Pharma Products Pvt. Ltd., Thayavur, India).

Conclusion

Due to its multifarious uses, it is a true "miracle tree". The *M. oleifera* Lam. is providing very good nutrition as protein essential elements supplements, but also a very good in curing and prevention of many diseases in human being. The leaves are extensively used for humankind but also very good cattle feed and handwash. The seed-powder can be used as water purifier, particularly for heavy metals and other impurities in water. The seed oil is also having high values as cosmetics, hair-skin care and as lubricants equivalent to oleic acids. Thus, due to multifarious uses of this tree India and state Government in particular take initiative to plant more and more trees in unutilized areas. India could easily fight against the problems of malnutrition, hunger, poverty, diseases, unemployment and edible oil export and earn lot of foreign exchange. More researches should be undertaken for its optimum production as a crop and its phytochemical should also be isolated for the synthesis of drugs for the utilization of humankind.

This plant truly appears to be a miracle tree having unlimited benefits for people and thus should be taken as a high quality gift of nature at very low price.

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Review Article

Genetic Diversity as Buffer in Biodiversity

Ashok Kumar Verma

Abstract

Almost every ecosystem maintains its own environmental insurance system. In order to maintain this system, an ecosystem needs three kinds of diversity: biological, genetic and functional. Biological diversity refers to the richness of species in a particular area; genetic diversity refers a way for a particular species to adapt itself to changing environments while functional diversity equates to the biophysical processes that happen within the area. One of the most important impacts of genetic diversity is that it acts as a buffer against the variability of environmental conditions particularly in the medium and long terms.

Keywords: Biological Diversity; Genetic Diversity; Ecosystem; Conservation; Values; Society.

Introduction

Biodiversity or biological diversity refers to the variety of life on Earth, including plants, animals, micro-organisms and the genes they contain. It simply means the existence of a wide variety of plant and animal species in their natural environments or the diversity of plant and animal life in a particular habitat.

Biodiversity is viewed as a measure of the relative diversity among organisms present in different ecosystems. In this definition, diversity includes variation within species and among species, and comparative diversity among ecosystems. Biodiversity may also be defined as the 'totality of genes, species, and ecosystems of a region'.

The Convention on Biological Diversity (Glowka *et al*, 1994) defines biodiversity as the variability among living organisms from all sources including, among other things, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are a part; this includes diversity within species, between species and of ecosystems.

A review of literature revealed that huge efforts have been taken and a number of scientists have worked a lot on biodiversity. Some of them are Kaushik *et al*, (2008), Odum (1971), Wilson (1988), Nair (1992), Bhatt (1997), Subba Rao (2001), Verma *et al*, (2015, 2016a, 2016b), Prakash *et al*, (2015, 2016a, 2016b), Verma (2016a, 2016b, 2016c, 2016d. 2017) etc.

The biodiversity is usually described at three levels and it has a large number of uses and values as well. In present discussion, author is trying to discuss genetic diversity as buffer and shock absorber of biodiversity in modern context.

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Three Basic Types of Biodiversity

The biodiversity is explored and described at three levels *namely*: ecosystem diversity, species diversity and genetic diversity. The ecosystem diversity is the diversity of habitats (place where an organism or a population of organisms naturally occurs), which include the different life forms within. Diversity at the level of community and ecosystem exists along 3 levels. First is alpha diversity (within community diversity), second is beta diversity (between

communities diversity) and the third is gamma diversity (diversity of the habitats over the total landscape or geographical area).

The species diversity refers to the variety of species within a region. It is the variability found within the population of a species or between different species of a community. The species is the real basic unit used to classify the organisms and its diversity is the most commonly used level for describing the biodiversity. It represents broadly the species richness and their abundance in a community. Species are therefore distinct units of diversity, each playing a specific role in the ecosystem. In nature, the number and kind of species, as well as the number of individuals per species vary, leading to greater diversity. The species are grouped together into families according to shared characteristics.

The genetic diversity is the diversity of the basic units of hereditary information (genes) within a species, which are passed from one generation to next. The genetic diversity results in variations hence the basic source of biodiversity and the amount of genetic variation is therefore the basis of speciation. The genetic diversity enables a population to adapt according to its environment hence important for natural selection. Genetic diversity within a species often increases with environmental variability but not all groups of animals have the same degree of genetic diversity. To conserve genetic diversity, different populations of a species must be conserved.

Existence Values of Biodiversity

Richard (2015) told that genetic diversity plays an important role in the survival and adaptability of a species. Its different values and uses include: consumptive use, productive use, social value, aesthetic value, scientific and evolutionary values etc. Moreover, biodiversity has ethical or existence value, which is based on the concept of '*Live and Let Live*'. It means biodiversity is valuable because if we want our human race to survive and continue then we must protect all biodiversity i.e. 'all life must be preserved'. If we want our human race to survive then we must protect all biodiversity because biodiversity has existence value from natural and ecological point of views.

According to Chris Maser (2009), almost every ecosystem maintains its own environmental insurance system, for which it needs three kinds of diversity: biological, genetic and functional. Biological diversity refers to the richness of species in a particular area; genetic diversity refers a way for a particular species to adapt itself to changing environments while functional diversity equates to the biophysical processes that happen within the area. One of the most important impacts of genetic diversity is that it acts as a buffer against the variability of environmental conditions particularly in the medium and long terms.

The living world has rich diversity of animals, plants and microbial life that appear to be well adapted according to the environment. This varied diversity must have to be maintained in order to mutual survival and existence of living beings. If a population of a species has a very diverse gene pool then there will be more variety in the traits of individuals of that population and consequently more traits for natural selection to act upon to select the fittest individuals to survive.

The biodiversity is being depleted by the loss and deterioration of habitats, over exploitation of resources, unprecedented climatic changes, pollution, diseases, cultivation shifting, poaching of wild life etc. Since the human beings are deriving all the benefits from biodiversity hence they should take proper care for the preservation of biodiversity in all its forms and good health as well as safety for the future generation.

Conclusion

An ecosystem is a set of life forms (biotic components) interacting with one another and with the non-living elements (abiotic components) of their environment. The ecosystem is therefore a community of organisms and their physical environment interacting together. As we lose species from existence, whether local or total or glocal, we lose not only their diversity of structure and function but also their genetic diversity. The loss of genetic diversity sooner or later leads in transforming complex ecosystems to so simple that they will lack the productivity and resilience to sustain us as a society.

Thus, biological diversity passed forward through genetic diversity that effectively maintains the functional diversity. The genetic diversity acts as a buffer against the variability of environmental conditions particularly in the medium and long terms. An ecosystem may be stable and able to respond positively to the disturbances in its own environment, to which it is adapted. Net result is that healthy environments can act as shock absorber in all types of disturbances.

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Book Review

Invertebrates

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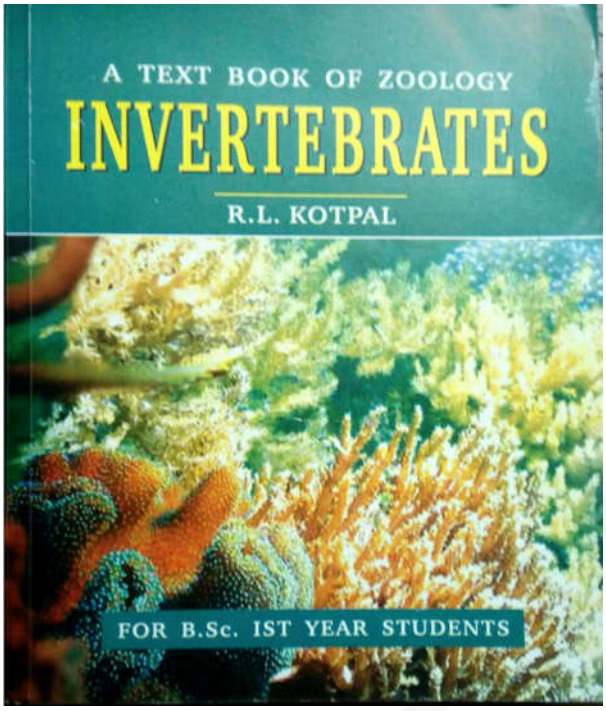
Professor R.L. Kotpal has written: A Text Book of Zoology "INVERTEBRATES" to develop a clear cut understanding in Zoology. Prof. Kotpal realized that many Indian universities have revised and reduced their syllabi in Invertebrates and therefore there was an urgent need to write and publish a book that fulfills the requirements of the students concerned. Due to this need, author and publisher decided to bring out an abridged edition of the book entitled 'A Text Book of Zoology: Invertebrates'. This book tells that how clear cut concepts can be developed regarding Invertebrate parts of Zoology.

The book entitled 'A Text Book of Zoology: Invertebrates' is published by Rastogi Publications, Shivaji Road Meerut-250002 (U.P.), India and its revised 10th edition came before during 2014- 2015. The revised edition of this book comprises 8+14+600= 622 pages. It contains 44 chapters of text matters supported by suitable diagrams and examples. This is followed by sufficient number of important descriptive questions and multiple choice type questions with answer of the same. Its author Prof. R.L. Kotpal is well known Academician and reputed Zoologist with International fame having several years of quality teaching and research experience.

The book 'Invertebrates' is written in five units, basically for first year students of B.Sc. offering Zoology. The first unit is Introductory having 2 chapters, second unit is of Subkingdom: Protozoa comprising 7 chapters, third unit is of Metazoa having single chapter, fourth unit is of Lower Invertebrates with 17 chapters and last unit is of Higher Invertebrates having 17 chapters.

Different Units with Chapters Including Key Points of this Book are as following:

1. *Introduction:* This first unit has 2 chapters *namely*: Diversity of Animals and The Invertebrates. In this unit, modern classification of animals with important features is given. It describes that what are Invertebrates and how they are different from Vertebrates. The subject matter is illustrated both by text matters and tables.
2. Subkingdom-Protozoa: This unit comprises 7 chapters *namely Euglena viridis, Trypanosoma gambiense, Plasmodium vivax, Paramecium caudatum, Monocystis*, Protozoa: Characters, Classification and Types and last chapter is Protozoa General Account. Life cycles of *Euglena, Trypanosoma, Plasmodium, Paramecium* and *Monocystis* are given in detail.
3. Subkingdom Metazoa: This unit has only one chapter Organization of Metazoa. It explains the metazoa, lower and higher metazoa, metazoan organization, symmetry and its significance, cephalization and polarity, metamerism, coelom, its origin, types and significance and different grades of organization.
4. Lower Invertebrates: It has 17 chapters *namely 1. Scypha (Sycon):* A syconoid sponge 2. Porifera: Characters, Classification and Types 3. Porifera : General Account 4. *Obelia*: A Sea fur 5. *Aurelia:* A Jelly Fish 6. Coelenterata: Characters, Classification and Types 7. Coelenterata: General account 8. Ctenophora 9. *Dugesia*: A Planarian 10. *Fasciola hepatica*: The Sheep Liverfluke 11. *Taenia solium*: The Pork Tapeworm 12. Platyhelminthes: Characters, Classification and Types 13. Superphylum: Aschelminthes (Nemathelminthes) 14. *Ancylostoma duodenale*: The Common Hookworm 15. *Wuchereria bancrofti*: The Filarialworm 16. Nematoda: Characters, Classification and Types 17. Helminthes: General Account. Life cycles of *Scypha, Obelia, Aurelia, Dugesia, Fasciola, Taenia, Ancylostoma* and *Wuchereria* are given in detail.
5. Higher Invertebrates: It comprises 17 chapters



namely 1. Neanthes (Nereis): A Clamworm 2. *Hirudinaria granulosa:* The Indian Cattle Leech 3. Annelida: Characters, Classification and Types 4. Annelida : General Account 5. *Palaemon (Macrobrachium malcolmsonii)*: The Indian Freshwater Prawn 6. *Palamnaeus:* the Indian Scorpion 7. *Apis:* The Honey Bee 8. Arthropoda: Characters, Classification and Types 9. Arthropoda: General account 10. *Peripatus* 11. *Pila*: An Apple Snail 12. *Unio (Lamellidens)*: Fresh Water Mussel 13. Mollusca: Characters, Classification and Types 14. Mollusca: General account 15. *Pentaceros*: Sea Pentagon or Oreaster 16. Echinodermata: Characters, Classification and Types 17. Echinodermata: General Account. In third and fourth chapters of this unit, characters and classification of annelids are given with suitable examples. Similarly, in the chapters 8th and 9th, characters and classification of arthropods and in 13th and 14th chapters, characters and classification of molluscs are given with suitable examples. Chapters 16th and 17th of this unit well illustrate the characters, classification of spiny skinned animals with suitable examples. Life cycles of *Nereis, Hirudinaria, Palaemon, Palamnaeus, Apis, Peripatus, Pila, Unio* and *Pentaceros* are given in detail. Economic importance of each phylum is given at the end of the chapter concerned.

The book entitled 'A Text Book of Zoology: Invertebrates' reviewed here is therefore an excellent, up-to-date and ultimate on the subject. Anyone can understand the invertebrate part of Zoology with the help of this book. It is well and nicely represented in simple language, which is very much useful to B.Sc. first year students offering Zoology as a subject. Simultaneously it is equally helpful to the students preparing for state and national level competitions.

Book Review: A TEXT BOOK OF ZOOLOGY 'INVERTEBRATES'

1. Name of book: A TEXT BOOK OF ZOOLOGY 'INVERTEBRATES'
2. Revised : 10th edition
3. Year : 2014-2015
4. ISBN: 81-7133-977-8
5. Pages: 8+14+600=622
6. Author: R. L. Kotpal
7. Price: Rs. 445/-
8. Publisher: Rastogi Publications, Shivaji Road, Meerut-250002 (U.P.), India

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3) Running title or short title not more than 50 characters;
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7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);
8) Source(s) of support in the form of grants, equipment, drugs, or all of these;
9) Acknowledgement, if any; and
10) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

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The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http://www.consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

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Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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