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Clinical evaluation of an Ayurvedic formulation in management of rheumatoid-arthritis

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Abstract

The aim of the study was to evaluate the efficacy of an Ayurvedic formulation in the management of Rheumatoid arthritis in order to establish the true efficacy of the formulation. A herbomineral formulation comprised of six medicinal plants - *Vitex negundo, Cyperus rotundus, Nyctenthes arbartristis, Simlex glabra Delphinium denudatum,* and *Withenia somnifera,* in combination of Maha yogaraj Guggulu Vaiswanar churna and Simhanada Guggulu was given as decoction to 140 patients aged between 12-60 years and of either sex for a period of one year. The diagnosis and evaluation of response of therapy was made according to subjective/ objective observations as per Performa prepared using score system in which points were assigned according to the involvement and severity of various findings which were recorded initially, periodically and at the end of the trial. Out of 140 subjects studied under trial, practically 97 subjects completed the treatment of which 39 (40.20%) subjects showed good response (relief of 75% and above) and 30(30.92%) subjects had fair response i.e. relief between 50% to 74% while 15 (15.46%) subjects experienced poor response and no response of the treatment was observed in 3 (3.09 %) subjects. This study demonstrated the Ayurvedic herbo-mineral formulation examined clinically could be used in management of the rheumatoid arthritis

Keywords: *Ayurvedic Research | Cure AIDS | Herbal Cure | Aroygadham*

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks synovial joints. About 1% of the world's population is affected by rheumatoid arthritis, women three times more often than men. The disease generally sets in the people between the ages of 40 and 50, but people of any age can be affected. The disease manifests in swelling, pain, redness, stiffness and warmth in the affected region and may lead to deformity of joint and restriction to mobility. As such, besides some painkillers, anti- inflammatory and immune-suppressant drugs with serious side effects,

there are no treatments which are considered completely effective and able to produce definite long-term relief.

One of the treatment alternatives drawing increasing attention lies in Ayurveda the traditional Indian system of medicine, that has been used in the Indian population for the treatment of such chronic diseases for several thousand years. The exact cause of RA is not known but this has been recognized as an auto immune disorder. In Ayurveda RA is described as 'AAMVAT' and its cause is very well explained[2]. The term Aamvat is derived from two terms Ama and Vata. Ama means formation of toxin that is produced by imbalanced body fire. The toxin ama is carried by imbalanced vata (one of the three energetic forces) and reaches the kapha (one of the three energetic forces) dominated places like joints etc. This toxin becomes sticky due to imbalanced doshas and blocks the vital channels which nourish the body.

The ama which gets harbored in joints acts like a foreign substance and triggers the immune system. This leads to inflammation of linings of joints. Ama is caused by imbalance of doshas resulted from indigestion due to Imbalanced foods and lifestyles, lowered body fire, sedentary work, over physical exertion involving lot of joint movements[3]. In recent past, trials of several therapies have been reported mostly with the Hetuvyadhiviparita drugs like sunthi Guggulu, Sunthi Guduchi, Vatagajankush Ras, Maharasnadhi Kwath & Yogaraj Guggulu, Amavatari Ras, Maharasnadhi Kwath[4,5] .Panhkarma treatment consisting of Snehana, Swedana, Virechana and Vasti has also been found to be significantly effective in most of the subjects.

Though according to modern medicine there is no specific cause of the RA, the concept of Ayurveda suggesting the production of ama or impaired metabolism invites the attention of researchers to combat disease by eliminating the causative factor. The treatment according to Ayurveda in addition to alleviation of the disease, it also aims at augmenting the process of digestion both at intestinal and cellular level. One of the authors (AR) is an Ayurvedic physician practicing Ayurveda for the last 28 years in his hospital 'Arogyadham'. He conducted a clinical trail on 150 subjects using a herbal formulation comprised of six medicinal plants - *Vitex negundo, Cyperus rotundus, Nyctenthes arbortristis, Simlex glabra, Delphinium denudatum,* and *Withania somnifera*, in combination of Maha yogaraj Guggulu Vaiswanar churna and Simhanada Guggulu, the drugs prescribed in Ayurveda for treatment of RA, to study the efficacy of the formulation in treatment of the RA and also studied the mode of action of the formulation based on the pharmacodynamic principles. Results of the study are reported herein.

Materials and Method

The subjects for the study were those visited Aroygadham Global Aids Research Foundation for treatment of RA. The nature, aim, procedures, and possible risks and benefits of the study were explained to the eligible subjects. Both verbal and written informed consents were obtained prior to the screening.

Inclusion criteria

The inclusion criteria were the following.

1. Age between 11 to 60 years
2. Sex- either sex
3. Chronicity between 6 to 5 weeks
4. Morning stiffness

5. Pain on motion or tenderness in at least one joint
6. Swelling of one joint
7. Swelling of at least one other joint
8. Symmetrical joint swelling
9. Subcutaneous nodules over bonny prominences
10. Typical **roentgenographic** changes which must show deminer-alizatio/ degenerative changes
11. Positive test of rheumatoid factor in serum
12. Synovial fluid a poor mucin clot with dilute acetic acid
13. Synovial histopathology consistent with rheumatoid arthritis
14. Characteristic histopathology of rheumatoid nodules evidenced by biopsy

Exclusion criteria

The exclusion criteria were the following.

1. Age below 11 and 60 years or above
2. Chronicity
3. Gout
4. Osteoarthritis
5. Tubercular arthritis
6. Gonorrhoeal arthritis
7. Arthritis with malignancy
8. Acute pyogenic arthritis
9. Psoriatic arthritis
10. Osteomyelitis
11. Rheumatic fever
12. Ankylosing spondylitis
13. Serious complications associated with any other systemic disease

Criteria for diagnosis and evaluation of response of therapy

The diagnosis and evaluation was made according to subjective/ objective observation as per proforma prepared using score system in which points were assigned according to the involvement and severity of various findings which were recorded initially, periodically and at the end of the trial (Table 1) and results of response of therapy were expressed as per the classification shown in Table 2.

The diet recommended / provided to the subjects in lunch was comprised of rice, pulse, vegetable curry and chapati.

A herbomineral formulation comprised of six medicinal plants - *Vitex negundo, Cyperus rotundus, Nyctenthes arbortristis, Simlex* ***Glabra*** **„** *Delphinium denudatum,* and *Withania somnifera,* in combination of Maha yogaraj Guggulu Vaiswanar churna and Simhanada Guggulu was given as decoction to 140 patients aged between 12-60 years and of either sex for a period of one year. In Maha yogaraj Guggulu Vaiswanar churna and Simhanada Guggulu total number of 35 ingredients existed out of which 27 (77.14%) were of plant origin while 8 (22.86%) were metals/minerals derivatives. The Decoction is to be taken Morning and Evening before Meals 2 times a day All 6 Herbs (5 gms each) mix together total 30gms divided means 15gms in Morning and evening to be taken as decoction half morning and evening at least for a year.

Results and Discussion

(a) Incidence of age: Observation regarding the incidence of age in Amavata patients

revealed highest number of incidence i.e. 31 (22.14%) in the age group of 21 to 30 years, 30 (21.42%) in the age group of 31-40, 27 (19.28%) in the age group of 41 to 50 years, 22 (15.71%) in the age group of 11 to 20 years, 21 (15%) in the age group of 51-60 while 9 (6.42%) subjects were in the age group of 61 and above.

(b) Incidence of sex: Regarding the incidence of sex in the subjects, females dominated the males, the proportion being 73 (52.14%) : 67 (47.85%).

(c) Chronicity: As regards the chronicity of illness highest number of patients i.e. 77(55%) had chronicity within 365 days, 21(51%) subjects between 366 to 730 days, equal number of 18 (12.85%) subjects between 731 to 1095 days and above 1461 days while 6(4.28%) subjects between 1096 to 1460 days.

(d) Involvement of joint: Study of the incidence of joint involvement revealed the highest number of 96 (68.57%) subjects afflicted with right knee joint followed by 92(65.71%) subjects being afflicted with the left knee joint. The other joints commonly involved were right ankle, Left MTP, I.P.T. of right hand, MTP joints of left hand, left ankle, right wrist and elbow, left wrist and elbow.

(e) Presenting signs/symptoms: Incidences of clinical signs and symptoms are given in Table 3. Swelling, pain, tenderness and morning stiffness were present in all the patients while 120 (85.71%) subjects had restriction of joint movement, 107 (76.42%) subjects had loss of appetite, 97 (69.28%) subjects had constipation and 63 (45%) subjects had anorexia. Incidences of loose motions and subcutaneous nodules were observed in 06 (4.28) and 01 (0.7) subjects, respectively.

Results in relation to age group, sex and chronicity are presented in Tables 3-5, respectively. Out of 140 subjects, 97 completed the treatment. Observation on the therapeutic effect of the drug showed good response in 39 (27.85%) subjects, fair response in 30 (21.42%) subjects while 15 (10.71%) subjects demonstrated poor response. The results in relation to sex as shown in table 3 indicate slightly more pronounced effect in case of females than the males. Data given in table 4 reveal that the highest subjects (10) showing good response were in the age between 41-50 years, 8 in the age between 31-40 years and 11-20 years, 7 in the age between 21-30 years, 05 in the age between 51-60 years followed by 01 in the age > 61 years. The highest subjects (10) showing fair response were in the age between 51-60 years followed by 06,05,04,04 and 01 in the age group between 11-20, 31-40, 21-30, 41-50 and >61 years, respectively. The % of the subjects having poor response was low in all age groups except 21-30 and 31-40 years. Only 03 subjects in the age group 11-20 (02) and 51-60 (01) years demonstrated no response.

Out of 140 subjects studied under trial, practically 97 subjects completed the treatment of which 39 (40.20%) subjects showed good response (relief of 75% and above) and 30(30.92%) subjects had fair response i.e. relief between 50% to 74% while 15 (15.46%) subjects experienced poor response and no response of the treatment was observed in 3 (3.09 %) subjects.

1. Subjective			
1.1 Morning stiffness points	**Score**		**Score**
Severe	06	**2.5 Restriction of joint movement**	
Moderate	04	Fully restricted	06
Mild	02	Partially restricted	03
Nil	00	Not restricted	00
1.2. **Pain on rest**		**2.6 Subcutaneous nodule**	
Severe	09	Present	02
Moderate	06	Nil	00
Mild	03	**2.7 Functional Status**	
Nil	00	Grade	06
2. Objective		Grade	04
2.1Pain in motion		Grade	02
Severe	09	Grade	00
Moderate	06	**2.8 Fever**	
Mild	03	Present	02
Nil	00	Absent	00
2.2. Swelling		**2.9 Elevated E.S.R. (first hour)**	
Severe	15	71mm or more	06
Moderate	10	41 mm -70 mm	04
Mild	05	20 mm - 40 mm	02
Nil	00	> - 20 mm	00
2.3 Tenderness		**3.0 Digestive impairment**	
G1	20	**3.1 Constipation**	
G2	15	Regularly	03
G 3	10	Frequently	02
G4	05	Occasionally	01
Nil	00	Nil	00
2.4 Muscle power		**3.2 Loss of appetite**	
G0	10	Appetite lost	02
G1	08	Appetite	01
G2	06	Normal	00
G 3	04	**3.3 Anorexia**	
G4	02	No inclination for food	02
Nil	00	Lesser inclination for food	01
		No anorexia	00
		3.4 Loose motions	
		Present	02
		Absent	00

Table 1: Criteria for diagnosis and evaluation of response of therapy

A. Good response	1. Presenting symptomatology of the disease as mentioned in the criteria for assessment.
	2. Laboratory parameters inclined towards normalcy.
B. Fair response	1. 50% and above relief in presenting clinical symptommatology of the disease as per criteria of assessment.
	2. 25% and above relief in presenting clinical symptommatology of the disease as per criteria of assessment.
	3. Significant improvement in laboratory parameters.
	4. Significant improvement in laboratory parameters
C. No response	1. No relief in syumptomatology or otherwise
D. Dropouts/LAMA Left against medical advice	1. Willful discontinuation of the treatment during the trial. 2. Development of any serious complication.
	3. Aggravation of the disease.
	4. Any pronounced toxicity of the drug.

Table 2 Classification of results

Ayurvedic Modus Operandi of Clinical Analysis

An effort was also made to analyze the pharmacodynamic principles of different ingredients of the formulations which was used in the combination of Maha yogaraj Guggulu, Vaiswanar churna and Simhanada Guggulu. The analysis on the presence of Rasa revealed that these 35 drugs have 62 constituents. Rasas out of which Katurasa dominates with 23(27.9%) followed by Tiktarasa being 16 (25.80%) and Kasayarasa being 10 (16.12%) 4(6.45%) and 3(4.83%) This indicates that this combination may render destruction of Ama and promote Deepama of Agni. The combination of drugs possesses 89 constituent Gunas out of which Laghu Guna dominates with 25 (28.08%) followed by Ruksha being 17 (19.10%) which are contradictory to the properties of ama and kapha The properties like snigdha being 16.85% is also significant of alleviation of vata. Next to these lies Tikshna Guna being 11.23% which is likely to act as srotasodhan. Other properties like Guru sita ushma sara sukshma and Pichhila also co exist to lesser extent. The distribution of virya as happened in these 35 drugs are 35 in total. Out of which Ushma virya is predominant being 68.57% followed by Sita being 22.85% while Anushnasita was 8.57% this model seems to be potent for Ama vata and kaphanasak and likely to alleviate the pain, improve the circulation and reduce the stiffness of the joints by absorbing accumulated tissue fluid. The vipaka of these 35 drugs also exist in a typical proportion as katu being 51.42% is likely to counteract the features of ama while madhura being 48.58% is the ideal end product of the drugs responsible for alleviation of vata. The individual Dosic action of the drugs occur in the proportion of Vatahara 59.61% followed by kaphakara as 34.06% while pittahara action was only 5.76% as such this makes a significant model for reversal of the disease process attributed

to Ama and vata. The other individual actions of the drugs as enumerated in the compendiums of Ayurvedic Materia Medica are 38 in total out of which Dipana accounts 28.94% followed by Vedanaamak /Sulahara 23.68 pachana and sothahara action both account for 18.42% while Amahara happens to be only 26.3% of proportion This model further corroborates the adaptability of this combination as both Hetuviparat and vyadhiviparia in case of aamvata.

Results	Male	Female	Total
Good response	18	21	39 (27.85%)
Fair response	13	17	30 (21.42%)
Poor response	06	09	15 (10.71%)
No response	02	01	03 (2.14%)
Drop out	28	25	53 **(37.85%)**

Table 3. Results in relation to sex

Results	**Age groups (in years)**						**Total**
	11-20	**21-30**	**31-40**	**41-50**	**51-60**	**61 & above**	
Good response	08	07	08	10	05	01	39 (27.85%)
Fair response	06	04	05	04	10	01	30 (21.42%)
Poor response	01	04	05	01	02	02	15 (10.71%)
No response	02	0	0	0	01	0	03(2.14%)
Drop out	07	14	12	12	03	05	53 (37.85%)

Table 4. Results in relation to age group

Results	**Chronicity (in days)**					**Total**
	0-365	366-730	731-1095	1096-1460	1461-above	
Good response	21	08	06	03	01	39 (27.85%)
Fair response	17	03	05	02	03	30 (21.42%)
Poor response	07	03	02	0	03	15 (10.71%)
No response	02	0	01	0	0	03(2.14%)
Drop out	20	07	04	01	11	53 **(37.85%)**

Table 5. Results in relation to chronicity

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Diversity of timber yielding plants found in different parts of district Tehri Garhwal, Uttarakhand, India

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Abstract

This research paper presents the timber yielding plants found in Chamba block and adjacent area of Tehri District. 37 (32 angiosperms and 05 gymnosperms) common timber yielding plants were found from different parts of the study area. The climatic conditions of the area of study was suitable for the vegetation wealth but due to weathering of rocks, deposition of soil particles and shifting of a number of population from different parts of Tehri Hydro Dam Project affected area, the environmental conditions become changed which is a bad signal for native plants of this area.

Keywords: *Timber yielding plants | Garhwal Himalayas | Tehri | Medicinal plants*

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Introduction

At present time timber yielding plants ruined next to food and fibres as the most widely used plant product. The range of Garhwal Himalaya covers almost the entire range of woody communities. The exploration of timber species was largely influenced by the accessibility; however, the demand of the prevalent economic activities often proved to be the ascendant force. The studies on the timber plants have been made by many workers in different parts of the world as well as India. Some important contribution were made by Balfour (1862), Watt (1908), Gamble (1922), Pearson and Brown (1932), Naidu (1934), Record and Hess (1944), Howard (1948), Troter (1960), Sagreiya (1967), Singh and Singh (1987), Dhaulakhandi (1996), and Dobhal (2003).

The study area is located near the New Tehri town and adjacent area at the elevation ranging from about 700 to 2600 m. The soil with high organic matter 5-15 % formed in temperate and cool humid region and low (1-3 %) in soil arid and semi-arid zones. The average rain-fall in the year was recorded 100-170 cm. while temperature between 20 °C-32 °C. During winter, temperature was 3-10 °C or may be below to 0°C.

Materials and Methods

Such studies on timber yielding plants will be conducted on different areas of Chamba block of Tehri district. Frequent field trip (April 2005 to March 2006) will be made to various parts of the study area to collect the specimens of timber plants and maintained in herbarium sheets. The standard methods of specimen collection, preservation and maintenance in the herbarium were followed (Jain and Rao 1977). Timber yielding plants collected were identified with the help of recent and relevant regional floras and comparing these specimens with authentically identified specimens preserved in herbaria of Forest Research Institute (DD), Botanical Survey of India, Northern Circle (BSD) and D.A.V. (P.G.) College at Dehradun. All the specimens have been deposited in the Herbarium of the Department of Botany, D.A.V. (P.G.) College, Dehradun. Their economic uses will be known by interviewing the natives and by consulting literature.

Result and Discussion

Following is the list of 37 important and common timber yielding plants (32 angiosperms and 05 gymnosperms) found in the study area (Table 1). They are arranged alphabetically according to their scientific names, families in parenthesis, vernacular names in Garhwali, herbarium and collector's initial, voucher number of specimens, habitats of plants and uses. Specimens of all species described here are preserved in the Herbarium of the Department of Botany, D.A.V. (P.G.) College, Dehradun.

A description of most common timber yielding trees which found in different localities of Chamba block and other parts of Tehri district discussed as below-

***Acacia arabica* Willd.** (Mimosaceae)

D.AV.-AD : 1866

Vernacular name : Babool

Wood - The sapwood is yellow-white in colour, heart wood is pinkish in colour. Hard wood is tough.

Economic uses: It is a very popular fuel wood used for carts, agricultural implements, beams, door frames, railway blocks, roofers, carving turnery.

***Acacia catechu* Willd** (Mimosaceae)

D.AV.-AD :1867

Vernacular name : Cathea

Wood - Yellow-white or red-brown in colour.

Economic uses: Used for packing cases, crates, clap furnitures, sleepers, paper, match boxes, vehicles etc.

***Abies pindrow* Spach.** (Pinaceae)

D.AV.-AD : 1868

Vernacular name : Fir

Wood - Sap wood is white or brown in colour and resinous in odour.

Economic uses: Used for packing cases, crates, cheap furniture's, sleepers, paper, match boxes, vehicles etc.

***Albizia procera* Benth.** (Mimosaceae)

D.AV.-AD : 1869

Vernacular name : Siris

Wood - The sap-wood is white, textured, hard and heavy strong. During seasoning, surface crakes and splitting develop in wood.

Economic uses: used in high class furnitures, table, desks, chairs, Elmira's, railway carriage, toys, floorings, carving turnery, cooperage etc.

***Bombex ceiba* DC.** (Bombaceaceae)

D.AV.-AD : 1873

Vernacular name : Semal

Wood - The wood is ceramic-white or pale-pink in colour. It is very light and soft.

Economic uses: It is universally used for making match boxes, match sticks, furnitures, boxes, packing cases, plywood etc.

***Cassia fistula* Linn.** (Caesalpiniaceae)

D.AV.- AD : 1874

Vernacular name : Amaltash

Wood - Heart wood is yellow, tough, hard and durable.

Economic uses: Used in agricultural implements, bark is used in tanning and dyeing.

***Cedrela toona* Roxb.** (Meliaceae)

D.AV.-AD : 1875

Vernacular name : Toon

Wood - The wood is pinkish-red, textured, lustrous, cedar like pleasant smell, strong durable, takes up a good finish and sprit polish.

Economic uses: The wood is extensively used for cheap furnitures, building purpose, tea and cigar boxes, carried items and thin plywood.

***Cedrus deodara* Loud.** (Pinaceae)

D.AV.-AD : 1876

Vernacular name : Deodara

Wood- Sap-wood is white; hart wood is light yellow-brown in colour, pungent smell, hard and heavy, contains volatile oil. The sap wood required antiseptic treatment. Developed easy timber to aired season, but the wood develop surface cracked and split during dry season.

Economic uses: Deodar wood is used for railway sleepers, house building, furnitures, posts, beams, floor, windows, doors, frames, packing cases. Ordinance Department uses the wood for boxes.

***Celtis australis* L.** (Urticaceae)

D.AV.-AD : 1877

Vernacular name : Kharik

Wood - Yellow-grey with irregular streaks of darker colour, tough, strong.

Economic uses: It is used for oars, whip-handles, churn-sticks etc.

***Dalbergia sissoo* Roxb.** (Papilionaceae)

D.AV.-AD : 1878

Vernacular name : Shishum

Wood - Golden-brown or dark brown in colour, hard and heavy, beautiful grains and figures, shock resistance, quit easy to saw and work.

Economic uses: The wood is used for making cabinets, high class furnitures, wheel, spokes, tools handles, toys, cotlegs, wagon parts, house construction, floorings, sports, boat making, cooperage etc.

***Emblica japonica* Roxb.** (Euphorbiaceae)

D.AV.-AD : 1879

Vernacular name : Amla

Wood - Red in colour, hard and close grained, durable under water.

Economic uses: The wood is used for agricultural implements. Bark and fruit used as medicine and tannin.

***Grewia oppositifolia* Roxb.** (Tiliaceae)

DAV-AD : 1880

Vernacular name : Bhimal

Wood - White in colour, tough and elastic.

Economic uses: The wood is used for oar-shafts, axe-bows, poles, agricultural implements etc.

***Haldina cordifolia* Hook. f.** (Rubiaceae)

DAV-AD : 1881

Vernacular name : Haldu

Wood - It has yellow coloured, hard and strong, small surface, cracks developed in seasoning, easy to sow and machine and take a good polish.

Economic uses: The wood is used for turning, carving, paneling, furniture's, toys, combs, brush-backs, rules, picture frames, pensile, roof boards, bath-room, kitchen filaments, railway carriage work, cigar boxes, plywood etc.

***Juglans regia* Linn.** (Juglandaceae)

DAV-AD : 1882

Vernacular name : Akhrot

Wood - It is greenish-brown in colour, light, strong and shock resistance, take fine polish, close grained.

Economic uses: The wood is used for making furnitures, gun-sticks, cabinets, carved items, toys, plywood.

***Lyonia ovalifolia* D.Don.** (Ericaceae)

DAV-AD : 1884

Vernacular name : Anyar

Wood - Light-brown and hard.

Economic uses: The wood is widely used for fuel and charcoal, for making agricultural implements.

***Mangifera indica* Linn.** (Anacardiaceae)

DAV-AD : 1887

Vernacular name : Aam

Wood - No distinct heart wood and sap wood, grey in colour, heavy and hard, not durable against termites and fungi, easy to sow and work used after treatment and preservatives.

Economic uses: It is used for making furnitures, tea-boxes, door, windows, agricultural implements, frame, plywood etc. It is also valuable for its fruit.

***Morus alba* Linn.** (Moraceae)

DAV-AD : 1888

Vernacular name : Shtut

Wood - Sap-wood is white in colour, hard and heavy, shock resistance, high strength.

Economic uses: The wood is used for furniture, hockey-sticks, tennis, badminton, cricket stamps, toys etc.

***Myrica esculenta* Thanb.** (Myricaceae)

DAV-AD : 1889

Vernacular name : Kaphal

Wood - Wood is grey in colour, hard, split and warp.

Economic uses: The wood is used as poison for fish, play implements.

***Pinus roxburghii* Roxb.** (Pinaceae)

DAV-AD : 1890

Vernacular name : Chir

Wood - The sap-wood is white, heart-wood is yellowish or light brown, resin abundant.

Economic uses: It is used for railway sleeper after treatment, packing cases, constructional work, for making light furniture etc.

***Pinus wallichiana* Wall.** (Pinaceae)

DAV-AD : 1891

Vernacular name : Kail

Wood - Slightly pinkish in colour, much hard and heavy, resin canal present, strong and durable, one of the best timbers of the Indian pines.

Economic uses: For making high class furniture, drawing boards, harmonium, sleepers, constructional work, doors, frames etc.

***Prunus cerasoides* Roxb.** (Rosaceae)

DAV-AD : 1892

Vernacular name : Paiyan

Wood - Heard wood is red, Hard, strong, pleasant smell.

Economic uses: The branches with shining bark are used for walking sticks.

***Quercus floribunda* Lindl.** (Fagaceae)

DAV-AD : 1894

Vernacular name : Moru

Wood - Heart wood is ed-grey in colour, very hard and uniform.

Economic uses: It is used for constructional work, building, agricultural implements, toys, axe-handles, walking-sticks.

***Quercus leucotrichophora* Roxb.** (Fagacea)

DAV-AD : 1895

Vernacular name : Banj

Wood - Heat wood is reddish-brown, very had, warps and split in seasoning.

Economic uses: Wood is used locally for building and for ploughs, agricultural implements etc. It is a good source of fuel.

***Rhododendron arboreum* Sm.** (Ericaceae)

DAV-AD : 1896

Vernacular name : Burans

Wood - soft, red-brown, warp and shrinked.

Economic uses: Used for fuel, building, gun-stocks, tool-handles etc.

***Syzygium cumini* Lam.** (Myrtaceae)

DAV-AD : 1898

Vernacular name : Jamun

Wood - Hard and heavy, reddish-brown in colour, resistant to water.

Economic uses: Used for construction work, house building, carts, wheels, agricultural implements, furniture, beams, boat making etc.

***Terminalia alata* W. & A.** (Combretaceae)

DAV-AD : 1899

Vernacular name : Asin

Wood - Hard and heavy, textured, red in colour.

Economic uses: Used for house building, boat building, agricultural implements, beams.

***Terminalia belirica* Roxb.** (Combretaceae)

DAV-AD : 1900

Vernacular name : Bahera

Wood - Light-grey or yellowish in colour, hard and coarse grained, not much durable.

Economic uses: It is used for various purposes, house building to packing cases. The fruit is very valuable.

***Zizyphus jujube* Lam.** (Rhamnaceae)

DAV-AD : 1901

Vernacular name : Ber

Wood - Hard, compact, tough, reddish-brown in colour.

Economic uses: Used for agricultural implements, good resource for fuel, charcoal, beams, walking sticks, cooperage industries.

Now a day most of timber yielding species extinguish due to increasing population and degradation of soil around the study area. The vegetation of the study area also influenced by construction work of Tehri Dam Project, and shifting of a number of population from different parts of Tehri District. The climate of the area of study was suitable for the vegetation wealth but due to weathering of rocks and deposition of soil particles, the environmental conditions become changed which is a bad signal for native plants of the area. Beside these timber plants *Acer oblongum* (Kirmola), *Alnus napalensis* (Utish), *Alstonia scholaris* (Satparni), *Holoptelia intigrifolia* (Papri), *Podocarpus* etc were present in very less amount. Commercially very little (e.g. *Pinus roxburghii* and *Cedrus deodara*) of timber yielding plants were used as timber because most of them had very less values of frequency.

Due to highly restriction of Forest Department of Uttarakhand the process of deforestation become reduced in this area. Although some gymnosperms like *Cedrus deodara* and *Pinus roxburghii* used by local people for different construction works after permission of Forest Department of Uttarakhand.

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Table 1. List of most common timber producing plants of district Tehri Garhwal

S. N.	Botanical Name	Family	S. N.	Botanical Name	Family
1	**Abies pindrow*	Pinaceae	20	*Madhuca indica*	Sapotaceae
2	*Acacia arabica*	Mimosaceae	21	*Mallotus philippensis*	Euphorbiaceae
3	*Acacia catechu*	Mimosaceae	22	*Mangifera indica*	Anacardiaceae
4	*Albizia procera*	Mimosaceae	23	*Morus alba*	Moraceae
5	*Alnus nepalensis*	Betulaceae	24	*Myrica esculenta*	Myricaceae
6	*Anogeissus acuminate*	Combretaceae	25	**Pinus roxburghi*	Pinaceae
7	*Anthocephalus cadamba*	Rubiaceae	26	**Pinus wallichiana*	Pinaceae
8	*Bombex ceiba*	Bombaceaceae	27	*Prunus cerasoides*	Rosaceae
9	*Cassia fistula*	Caesalpiniaceae	28	*Pyrus pashia*	Rosaceae
10	*Cedrela toon*	Meliaceae	29	*Quercus floribunda*	Fagaceae
11	**Cedrus deodara*	Pinaceae	30	*Q. leucotrichophora*	Fagaceae
12	*Celtis australis*	Urticaceae	31	*Rhododendron arboreum*	Ericaceae
13	*Dalbergia sissoo*	Papilionaceae	32	*Sapium insigne*	Euphorbiaceae
14	*Emblica japonica*	Euphorbiaceae	33	*Shorea robusta*	Dipterocarpaceae
15	*Grewia oppositifolia*	Tiliace	34	*Syzygium cumini*	Myrtaceae
16	*Haldina cordifolia*	Rubiaceae	35	*Terminalia alata*	Combretaceae
17	*Juglans regia*	Juglandaceae	36	*Terminalia belirica*	Combretaceae
18	**Juniperus* sp.	Cupressaceae	37	*Zizyphus jujuba*	Rhamnaceae
19	*Lyonia ovalifolia*	Ericaceae			

*Plants belonging to gymnosperms

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Role of Organic Nutrients on the yield of *Ammi majus* L.

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Abstract

Majority population of developing countries still rely on plant based traditional drugs. Cultivation of medicinal plants for their products is in great demand especially due to high cost of their products. The present work is focused on improving the living standard of poor farmers by introducing most effective and profitable farming of medicinal herbs without discontinuing their traditional farming. *Ammi majus* L. a member of family Apiaceae has several medicinal properties. Its seeds have contraceptive and diuretic properties.It is mainly used in the treatment of vitiligo and psoriasis. It is also used as tonic or in the treatment of asthama and angina. The present demand of its seeds in the world market is worth for about 14 billion US dollars per year. Over exploitation of non-cultivated medicinal plants has become a threat to biodiversity in the forest areas in India. The seeds of *Ammi majus* L. were obtained from Hamdard University, Delhi were cultivated in farm land as well as Hislop college experimental field with control and treatment of various nutrient types. The present work has proved that this plant can be cultivated from February to May in the Vidarbha region of Maharashtra, India. The organic farming techniques have proven to be better yielding.

Keywords: *Medicinal Plants* | *Ammi majus* L., | *vitiligo* | *psoriasis* | *contraceptive* | diuretic.

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Introduction

The plant *Ammi majus* L. is a native of Nile Delta of Egypt. In India, *Ammi majus* L. was introduced in forest research institute, Dehradun in 1955 through the efforts of UNESCO for its medicinal & ornamental value (Bradu & Atal,1970;Singh,1963 and Umrao Singh et al.1982). Since then, its experimental cultivation has been tried in several parts of India including Jammu, Dehradun, Mumbai, Chennai, Delhi and Punjab.

The plant is used for the treatment of leucoderma & psoriasis (Anup Kumar, 1988 and Hansen, 1979). It has been recommended as a diuretic, expectorant & useful in Jaundice (Khan & Rehman, 1985 and Lal, 1977). The

fruits are used in vitiligo & also in the formation of suntan lotion (Anonymous, 1985, 1986). The essential oil from *Ammi majus* L. seed has been extracted by Ashraf *et al.* in 1972 who also studied its quality compound for the first time.

The seed is contraceptive, diuretic and tonic. An infusion is used to calm the digestive system, whilst it is also used in the treatment of asthma and angina. Its decoction is also used as a gargle in the treatment of toothache. It was an Egyptian professor Abdel Monem. El Mofty, who observed plants used in Egyptian folk medicine (e.g *Ammi majus* L.*)* and began the development of modern photochemotherapy (PUVA) for vitiligo and psoriasis. Klaber (1942) introduced the term phytophotodermatitis to emphasize the necessity of plants and light to cause the reaction. Duke (1988) have reported that furocoumarins have bactericidal, fungicidal, insecticidal, larvicidal, moluscicidial, nematicidal, ovicidal, virincidal and herbicidal activities.

Ammi majus L. is an important medicinal perennial herb belonging to family – Apiaceae. Its common name is Aatrilal, bishops weed, bullwort, False queen anne's lace, lace flower or mayweed. It has a striated subglaucous stems, leaves acute, serrulate, alternate, bipinnate & lobes oblong. Inflorescence a compound umbel, flowers bisexual, polygamous, bracteate, calyx teeth small, petals obovate, stamens epigynous, ovary inferior , two locular, stigma capitate. The plant prefers bright light, sandy medium (Loamy) or heavy (Clay) soils. The plant prefers acid, neutral or basic (Alkaline) soils. It can grow in semi shade (Light woodland) or no shade. It requires moist soil.

Material and Methods

Preparation of Experiment Field

The experimental field of Hislop College and a local farm, about 18 km away from the city were selected for the study. The field was ploughed to clear off the weeds and also for solarisation of the underneath soil layer to get rid off the unwanted soil fungal flora. The soil was mixed with organic compost manure. In local farm the field was divided in to 5 plots, each plot 100 ft. long and 5 ft. wide (500 sq. ft.). Due to lack of sufficient area the experimental field of the college was divided in to 5 parts each having length 5 ft. and width1 ft. Sufficient gap between each plot was given to prevent the influence of a particular treatment to the neighbouring plots.

Seed collection and sowing

The seeds of *Ammi majus* L. were obtained from Hamdard University, Hamdard Nagar, New Delhi. They were cleaned with sieve and weight of 1000 seeds was obtained by digital single pan balance in the laboratory. On the basis of weight, packets of 1000 seeds were made for the convenience in sowing in the farm. 1000 seeds/plot were sown in the farm while 10 seeds/plot were sown in the college experimental field. The seeds are sown at a depth of about 1.5mm-2mm & at a distance of 1 foot between each other. After germination thining and transplanting of the seedling was carried out in a manner that 500 strong plants were left in each plot while 5 plants/plot remained in the college field. Regular watering was done as per the need to keep the soil with sufficiently moistured. Deweeding and hoeing was done twice a month to avoid the problems created by weeds and also to make the soil soft with sufficient aeration.

Manures

Nutrient solution

An organic nutrient solution was prepared by fermenting fresh cowdung with neem oil cake. 200 liters of water with 5kg cowdung and 250 grams of neem oil cake were kept in a plastic drum. 2 gm of urea was added as a stimulant for bacterial fermentation. The fermentor drum was kept in optimum conditions of temperature i.e. 20-30 ºC for 15 days. The medium was stirred regularly for better fermentation. The supernatant liquid was taken as stock solution which was further diluted to 10 times with water before giving to the plants. The stock solution was diluted to 20 times for the use of foliar spray to the plants.

Vermi-compost

Vermi-compost was prepared in the college composting unit. It is of 40 ft. by 20 ft. in size and is divided in to 4 compartments by brick partition with holes to connect each other. The degradable garden waste was added to one compartment daily and cowdung occasionally till the compartment became full. Earth worms were introduced in to the compartment along with cowdung when the compartment was half full. A layer of about 1 ft. thick cowdung was added at the top and covered with a jute sheet. Required moisture was maintained by spraying water daily. Same process was repeated in each compartment one by one. As after about 70 to 80 days the composting process gets over, the water spraying was stopped so that Earth worms automatically move into the next compartment where moisture and semi degraded organic matter are available.

Inorganic fertilizers

The inorganic fertilizers were purchased from the market. They are Urea, Sterameal and DAP. Each plot with 480 sq.ft. size was given 100 gm Urea,10 gm Sterameal and 50 gm DAP twice during the crop period. Each 5 sq. ft. plot in the college was given 10 gm Urea, 1 gm Sterameal and 5 gm DAP twice during the crop period.

Sowing of the seeds was done on 12th Feb 2010.Thining, hoeing and transplanting was done after about two weeks of sowing (from 25th February to 1st March) in all experimental plots.Out of 5 plots 1st plot was kept as control. While other four plots were given the 1st dose of nutrients after about three weeks of sowing (on 5th March 2010) in the farm land. Different types of nutritional combinations used are: A) Control without any additional nutrition, B)Diluted nutrient solution, C) Vermi-compost, D) Vermi-compost and nutrient solution, E)Inorganic nutrient solution. Similar treatments were also applied in the college experimental field about three weeks after sowing of seed i.e. on 6th March 2010.Foliar spray of nutrient solution was given after four weeks *i.e.* 10th March 2010 to 'B' compartment only. The second dose of nutrients was given from 2nd and 3rd April 2010 in the same manner after a period of eight weeks since sowing.

About 70% germination was observed both in the farm land and the college field. About 500 plants were maintained in each plot of farm land while only 5 plants each were raised in college field (Plate-1 & 2). The rest were removed by thining and transplanting. Two doses of manures were given within a gap of four weeks. Flowering occurred after about eight weeks of sowing(7th April 2010, Plate-3 & 4) and fruits were matured after about twelve weeks of sowing(5th May 2010).The fruits were plucked and collected in 5 different polythene bags separately from farm land and college field from each plot. They

Plate -1
Vegetative Phase (Control)

Plate - 2
Vegetatative Phase (Vermicompost+Nutrient)

Plate - 3
Flowering Phase (Control)

Plate - 4
Flowering Phase (Vermicompost+Nutrient)

were allowed to dry in the lab by spreading it over newspaper and covered with net cloth for 15 days. Majority of the fruits were splited and rest were broken by soft grinding by hand. They were cleaned by a sieve and then packed in separate transparent polythene bags. The weight of 1000 seeds and total weight of seeds was measured. The results are depicted in Table: 1 for farmland and Table: 2 for the experimental field.

Result and Discussion

In the present investigation the effectiveness of four different types of manure/ nutrient treatement on the seed yield & seed weight of *Ammi majus* L. was determined. The findings are depicted in Table:1 & Table:2 and figures:1 & 2. On perusal of data given in Table:1 & 2, it becomes obvious that the nutrient type T4 (Vermicompost + nutrient solution) has excelled over other treatment

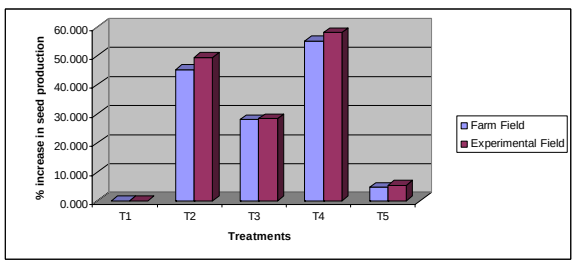

Figure 1: Role of Organic Nutrients on the seed production of *Ammi majus* L.

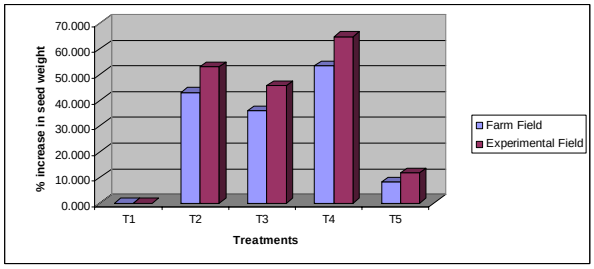

Figure 2: Role of Organic Nutrients on the seed weight of *Ammi majus* L.

type both in terms of seed yield & seed weight. It has exhibited an accretion of about 55% in the seed yield in farm field & about 58% in the Hislop College experimental field. Similarly, an increase of about 53% & 65% is recorded in seed weight from the yield of farm field & Hislop College experimental field respectively.

The overall pattern of effectiveness of different nutrient/manuring combinations in descending order can be expressed as follows:

T4 > T2 > T3 > T5 >T1 where,

T1 stands for Control, T2 for Nutrient Solution, T3 for Vermicompost, T4 for Vermicompost + Nutrient & T5 for Inorganic solution.

It is worth mentioning that better seed yield & seed weight has comparatively been recorded from Hislop College experimental field. This could be attributed to soil quality (garden soil) & moisture availability at this experimental site.

Many scholars have worked on the cultivation techniques of *Ammi majus* L. (Bradu *et al.*, 1970; Kumar, A. 1988; Singh V.P., 1963; Sobti *et al.*, 1978.) The present experiments were carried out during the month of February to May 2010. Many research workers have suggested different sowing timing for *Ammi majus* L. cultivation in India.

Panda (2002) has stated that an ideal time for direct sowing of *Ammi majus* L. in the field is

September whereas Singh & co-workers 1963 had recommended 30th October as the most suitable date for sowing of *Ammi majus* L. at Chakroli (Jammu). They have observed that sowing beyond this date cause significant reduction in the seed yield. These findings are in concurrence with the timing of Duhan and co-workers cf. Panda (2004).

S. No.	Treatment	Average Yield Per Plant	Total Weight of Crop (Seeds)	Percent Increase in Seed Production	Weight of 1000 Seeds	Percent Accretion in Seed Weight
1	Control (T1)	8.140 gm	4070.10 gm	-----	0.3650 gm	----
2	Nutrient solution(T2)	11.822 gm	5911.20 gm	45.235%	0.5220 gm	43.013%
3	Vermicompost(T3)	10.413 gm	5206.80 gm	27.98%	0.4960 gm	35.89%
4	Vermicompost + Nutrient (T4)	12.625 gm	6312.60 gm	55.096%	0.5600 gm	53.424%
5	Inorganic (T5)	8.520 gm	4260.20 gm	4.67%	0.3950 gm	8.219%

Table 1: Role of Organic Nutrients on the yield of *Ammi majus* L. (Farm Field)

S. No.	Treatment	Average Yield Per Plant	Total Weight of Crop (Seeds)	Percent Increase in Seed Production	Weight of 1000 Seeds	Percent Accretion in Seed Weight
1	Control (T1)	0.776 gm	3.880 gm	-----	0.340 gm	----
2	Nutrient solution(T2)	1.16 gm	5.800 gm	49.484%	0.520 gm	52.941%
3	Vermicompost(T3)	0.996 gm	4,980 gm	28.350%	0.495 gm	45.58%
4	Vermicompost + Nutrient (T4)	1.226 gm	6.130 gm	57.989%	0.560 gm	64.705%
5	Inorganic (T5)	0.818 gm	4.090 gm	5.412%	0.380 gm	11.764%

Table 2: Role of Organic Nutrients on the yield of *Ammi majus* L. (Experimental Field)

As per Panda (2002) the crop of *Ammi majus* L. on an average yield 12 q/ha of dry seeds. A yield of 1375 kg/ha has been obtained under experimental conditions & 900-1200 kgs/ha under large scale cultivation under Jammu Condition. In Palampur Baijnath area the yield of just 600 kg/ha has been obtained. In the present study a maximum yield of 600 kg/ha has been recorded using organic farming technique during off season months. A better yield could be expected during the optimum growing season between October-May as suggested by various worker (Duke 1988; Panda 2002, 2004; Singh 1963-1983).

As mentioned earlier, the combination treatment of nutritent solution and vermicompost together gives the best possible yield. Thus, one can very rightly go for organic farming in the cultivation of *Ammi majus* L. in Vidarbha region of Maharashtra state where maximum number of farmers suicides occurred due to failure of other crop & economic hardship.

The yield obtained from this research during February to May 2010 that too from the control indicates that the crop of *Ammi majus* L. can be used as a summer crop in the local climatic conditions after the harvesting of the regular crop. The organic farming methods

tried can be used to increase the yield remarkably and at the same time maintaining the fertility of the soil. It will definitely boost the economic condition of our poor farmers in Vidarbha region.

Conclusion

The present research work on cultivation of *Ammi majus* L. indicates that this medicinal herb can be grown as an additional summer crop after the harvesting of regular crops which will be over by February. The present study has revealed the importance of nutrients to enhance the production of this crop. Thus, it can be concluded that the organic farming will help to increase the productivity of this crop without compromising quality. Total organic farming of *Ammi majus* L. can yield better result in production without affecting the health of the soil. The production of about 600 kg. per acre will fetch between Rs.10 lacs to 11 lacs in the open market. This will boost the economy, help to improve earning potential of the farmers & enhance their living conditions alongwith earning valuable foreign exchange for the country.

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Determination of structure of new anthraquinone from the flowers of Marigold from Temperate zone of Garhwal Himalaya of Uttarakhand

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Abstract

The plant of marigold (Genda) is native of Mexico but this herbaceous plant is widely cultivated in all the parts of our contrary for commercial purpose and also grown in the gardens as an ornamentally. The botanical name of marigold is *Tagetes erecta* which belongs to family Asteraceae. The flowers are the source of yellow dye, which is commonly used to dye cotton. The present study related with investigation as isolation and structure of a new anthraquinone together with β-Sitosterol and 6,7-dimethoxy xanthopurpurin. The compound was determinate with the help chemicals and of spectral studies and comparison with authentic sample and reported data (Potter and Thomas 1995).

Keywords: *Marigold* | *Temperate zone* | *Garhwal Himalaya* | *Anthraquinone*

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Introduction

Marigold is erect annual aromatic herb which has simple leaves, yellow/orange homogamous flowers. Besides the dyes, the pastes of flowers were also applied as medicinally on wound and cuts (Gaur, 1999). *Tagetes erecta* and *Tagetes paluta* are commonly found in tropical as well as temperate zones of Himalaya. Principle constituents isolated from flowers of *Tagetes minuta* are anthocyanins and its derivatives (Prakash Rao and Putlano 2000). Some long chain fatty acids, aromatic hydrocarbons and phenyl acetaldehydes are extracted from floral extract of *Tagetes erecta* (Chawdhury 2001). The present experimental studies explain the isolation and structure of a new anthraquinone glycoside together with β-Sitosterol and 6,7-dimethoxy xanthopurpurin.

Materials and Methods

Collection of plant material

The flowers of Genda were collected from the different parts of Tehri district of Uttarakhand during the month of August-September 2000. The plant was identified with the help of voucher specimen of Herbarium of

Department of Botany, D.A.V. (P.G.) College Dehradun.

Extraction and isolation

The air-dried and coarsely powered flowers of the plant were defatted with light petroleum in a soxhlet. The defatted mass was exhaustively extracted repeatedly with 90 % aqueous EtOH, until the extractive became colourless. All the extracts were mixed and concentrated under reduced pressure using rotatory vacuum evaporator. The concentrated extract was adsorbed on Silica gel and fractionated through column chromatography using the solvent system of chloroform-methanol (95:5). The polarity of solvent was gradually increased by addition of methanol. Repeated column chromatography afforded compounds PT-1, PT-2, and PT-3 with some other inseparable compounds.

Results and Discussion

The ethanolic extract of flowers of *Tagetes erecta* on repeated column chromatography over silica gel afforded compounds PT-1, PT-2 and PT-3. Compounds PT-2 and PT-3 were identified as β-Sitosterol and 6,7-dimethoxy xanthopurpurin by comparison with authentic sample and reported data (Potter and Thomas 1995).

Compound: PT-1

It was crystallized from methanol as crystalline solid. On the basis of elemental analysis and molecular weight determination its molecular formula was deduced as $C_{26}H_{28}O_{14}$. The molecular weight of compound was found to be molecular weight: 564 amu, from FAB-MS. The UV spectrum showed absorption at 218, 225, 305 and 315. Its IR spectrum displayed a characteristic band of chelated carbonyl group at 1660 cm^{-1}. The ^{13}C-NMR spectrum of compound showed the presence of 26 carbon atoms. The assignment of all proton and carbon were achieved by ^{1}H-^{1}H-HOMO (COSY) and inverse ^{13}C-NMR data, which showed the anthraquinone skeleton of compound. The anomeric proton of compound exhibit doublet at δ 5.8(J=7.2 Hz) anomeric proton of β-linked D-glucose. A doublet at δ 4.62(J=2.0 Hz) were assigned for α-linked xylose. The downfield signal at δ 142.2, 142.8, 151.2, 158.1 were assigned for substitution at C-1, C-2, C-6 and C-8 carbon atoms, C-8 found to be glycosilated. Thus on the basis of above data PT-1 was identified as Anthraquinone 1,6-dihydroxy 2-methyl 8-O-β-D-glucopyranosyl (1→6)-α-L-xylopyranoside (Figure-1). It was further confirmed by comparison of its data with that of reported compound (Thomson 1971 and Wijnsma 1986).

Acknowledgments

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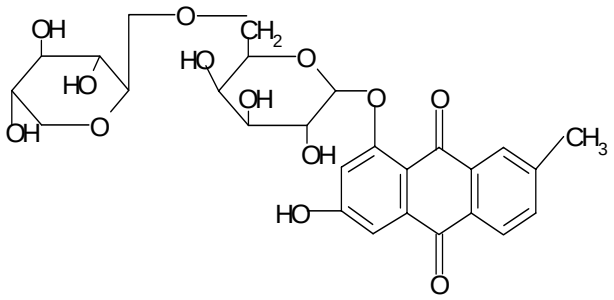
Figure-1



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The bioelectronics in exploration of chemicals, metals & other resources from geo-matters of earth under radioactivity and mrf-sensitization of medicinal plants

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Abstract

The medicinal plants are playing their vital role in maintaining human survival and environmental conservation by curing of creatures including stone anatomy as defense force of nature. The vigorous biochemical activities on the part of medicinal plants to face any crisis of climate and culture keeps them as smartest entity of matter, since they endeavor to maintain all the imbalances imposed upon by individual or collective in disciplines of different components of nature. The prevailing crisis on the globe in the shape of man-made and natural calamities of future millennium in the shapes of atomic war and the global warming menacing the whole creation of nature within a few years could resolved, if we sensitize these medicinal plants with vigorous bio-electronic capability growing their amphibiotic role, globally. One may wonder that quite incongruous to mobile intelligent human beings how a localized dump, deaf medicinal plant can be endowed with such a great artificial intelligence? Really, medicinal plants are much smartest to other plants and human beings too in the sense that they maintain an equilibrated transition phase between anatomic and un-anatomic matters as invaluable cosmic gift of nature. Their well known localized role as feeder and curer could vitalized by their extended duty against external invasions like Anthrax or Biological weapons provided that we consolidate them Bio-Electronically by the appropriate physical interactions such as Radioactivity-MRF-sensitizations. The Radioactivity - MRF-excitations of plants are strengthening their Photosynthesis like characters e.g. the absorption and the fluorescent bands within which photosynthesis occurs are widened due to such interactions. The proposed attempt is the modest endeavor in the direction of metallic exploration by promoting electroplating under MRF-sensitization of plants and thus restricting the inefficient costly and unnatural surface mining Geo-Technological processes imposing tremendous burden upon the environment.

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Keywords: *Bioelectronics* | *Radioactivity - MRF-Sensitization* | *Medicinal-Plants*

Introduction

The medicinal plants are sharing their vital responsibility in equilibrating the environmental conservation by ordering the unwanted collision of micro-constituents of bioprocess. Treating medicinal plants to be intermittent between bio-species and the geo-anatomy possessing electronic band structure type behaviours under going electrical conduction processes both with dc as well as the low and high frequency alternating currents like those on ion conducting doped crystals, glasses, polymers and amorphous semiconductors[1] had been tried. The nonlinear and chaotic oscillations in these materials[2,3]under the influence of transverse magnetic field H namely the dynamic Hall-Effect[4] had been extended to study the medicinal plants Cathranthus Roseus[5] and Alloy Veera[6] under MRF-perturbations. The magnetic field H along with the radiofrequency excitations when imposed upon the medicinal plants their electrical as well as magneto-conductivity both found to vary profoundly. The electrical conductivity σ_0 in these species under MRF-perturbations is switched on to in the range 100-1000 $\Omega^{-1}cm^{-1}$which is a clear cut distinguishing feature of them to be undergoing a phase transition to the fast ionic conductors (FICs)[7-9]. The fast ionic conduction imposed in medicinal plants under MRF-perturbation allow them to behave like good liquid electrolytes and an electronic electrode-net working may impart electrolysis for exploring the desired metallic ions under appropriate Radioactivity-MRF-dosage. The radioactivity exposure of the sample has been made by using α-particle source radionuclide $_{95}Am^{241}$ dose 100mC.

Theoretical Review

The application of electric field on biological system results in a net effective movement of positive charge in the shape of metallic ions towards the cathode. If the conduction occurs predominantly due to a single ionic species (under optimized MRF-dose) the electrical conductivity is given by:

$$\sigma = n\,(Ze)\,\mu \qquad (1)$$

Where; n = concentration of charge carriers, Ze = charge on the ion and μ the mobility of the ion. In the conventional Bio-FIC due to MRF-sensitization, the concentration of charge carriers may be given as follows;

$$n = n_0\, e^{-Ed/kT} \qquad (2)$$

Where; n = number of positive and negative ion vacancies, $n/n_0 = \beta$ = fraction of mobile ions and $E_d = \varphi/2$ i.e. φ = formation energy of ionic pair in bio-system. If E_d is large e.g. as in Na Cl ($E_d \sim 1eV$), n/n_0 is very small. In diffusion process through bio-systems, ions vibrating with a positive energy well of energy Em at characteristic frequency ν_0 may be assumed to make isolated jumps. Diffusion constant D depends on geometrical factor α. Assuming Boltzmann statistics applicable to the system, it may be shown that

$$D = \alpha\, d^2 \nu_0\, e^{-Em/kT} \qquad (3)$$

Em= Activation energy for migration. Nernst-Einstein equation yields:

$$\mu kT = Ze\,D$$

$$D = \mu kT/Ze \qquad (4)$$

From equations (3) and (4) one may have;

$$\mu = [(\alpha\, d^2 \nu_0\, Ze)/kT]\, e^{-Em/kT} \qquad (5)$$

On using equation (1) and (5) in equation (2) one may have

$$\sigma = [(n_0 (Ze)^2 \alpha\, d^2\, \nu_0)/kT]\, e^{-\{Em+Ed\}/kT} \qquad (6)$$

In MRF-stimulated Bio-FIC character: one may observe (a) highly ordered structural arrays which may be in the form of Tunnels, Layers or Three Dimensional Arrays generally localized on one of the ion sub-lattices. (b) highly disordered complementary sub-lattice in which the number of equivalent sites is greater than the number of available ions to fill them. (c) second feature providing the exceptionally high carrier concentration revealing Liquid-Like behaviour. (d) highly ordered bio-lattice system serves as stable frame work for mobile ions (e) the concentrations of additives from the environment may also heavily dope bio-systems and the degree of defects (contamination) depends upon impurity association. (f) as mobile ion sub-lattice is expected to be disordered under MRF-perturbation, high defect concentration may be present without thermal generation, therefore β may be nearly temperature independent and much larger than in normal ionic conductors and their activation energy will be only Em that of migration alone and thus

$$\sigma = [\{n_0 \beta \alpha d^2 \nu_0 (Ze)^2\}/kT]\, e^{-Em/kT} \qquad (7)$$

The constants in this equation are not known; a priori and customarily more general form is given by Arrhenius-like Temperature dependent equation:

$$\sigma = (\sigma_0/T)\, e^{-E/kT} \qquad (8)$$

This type of equation is satisfied by a vast majority of ionic conductors having crystalline, glassy, geo-rock/ minerals and micro-soft materials having bio-structures. For FICs the σ_0 = 100-1000 $\Omega^{-1}cm^{-1}$ which in simple ionic conductors have $\sigma_0 \sim 10^6\ \Omega^{-1}cm^{-1}$.

Experimental analysis

The Radioactivity-MRF-sensitization of Alloy Veera has been made[6]and experimental results have been shown in figures 1 & 2 along with computed data 3,4,5,6,7,8,9 below:

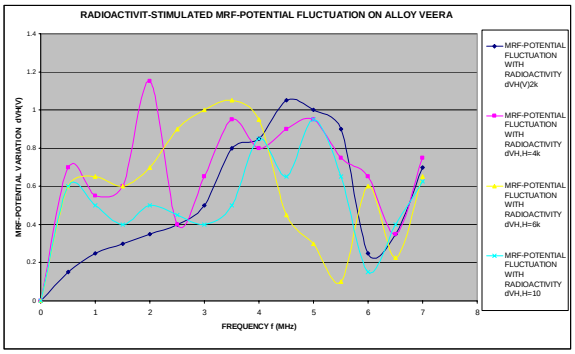

Figure 1.

Radioactivity imposed MRF-Magneto-Potential records at magnetic fields H=2,4,6,10 kG depict the peak characterization of bio-matter within *Alloy Veera* and that is due to heavy chemo-ionic condensation imposed upon by these external interactions which in natural growth of plants lie in very dilute state having very slow curing against diseases. For H=2 kG their exist two peaks, sharp one at f=3.2MHz having peak value=0.82 Volt and blurred one at f=4.6MHz having 1.3 volt magnitude. For H=4kG, 4-sharp peaks at f=0.6,2,3.5,4.75 MHz having peak values 0.71,1.1,0.95,0.9 V respectively have been observed.

For H=6000Gauss, 3-sharp peaks at f=0.8,3.5,6MHz having 0.63,1.1,0.6V magnitudes and for H=10kG, 4-sharp peaks at f=0.7,2,4.1,5.2 having magnitudes of 0.61,0.5,0.83, 0.95 respectively are observable. All these peaks having different half widths have been constituted by a variety of chemo-ionic condensations both

qualitatively as well as quantitatively. This new way of chemical characterization without using any prevailing chemical or spectroscopic methods may be regarded as MRF-Imaging[13] quite independent of stone or bio-anatomy both accepted gods & goddesses in Indian Ayurvedic philosophy.

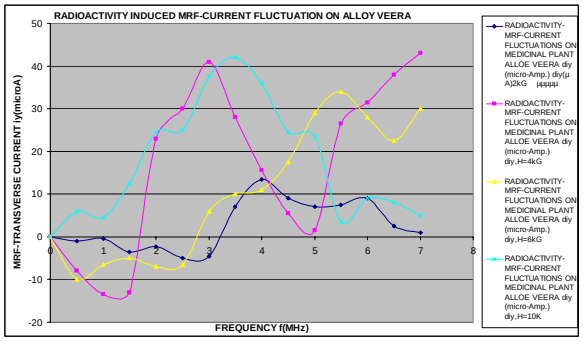

FIGURE- 2

Figure-2 is depicting MR-stimulated transverse electric currents in medicinal plant Alloy Veera which is due to only a fraction of mobile ions out of those absorbed from earth in the shape of natural food. The pronounced peaks for H=2000, 4000,6000,10000 Gauss lie at f=4,3,5.5,3.5 MHz having 13,41,34,43 micro-Ampere amplitudes. A number of secondary peaks of relatively lower magnitudes are also observable at different frequencies whose ionic-dynamics may also be computed. Figure-3 is representing MRF-dependent typical FIC-resistivity trends on Alloy Veera. The sudden rise in resistance between frequency range f=0.5-1.5 MHz to reach climax values Rmax=325kΩ, 310kΩ,225kΩ,185kΩ at H=2,4,6,10kG & f=1.5,0.56,0.65,0.8 respectively and then decreases exponentially with a few harmonics till it reaches minimum values at f=7MHz i.e. Rmin=50kΩ at H=2,10kG & Rmin~25kΩ., at H=4,6kG respectively. Some of these trends seems to obey Rmax.fmin=constant like black body radiation law Tmax . λmin=constant, λ being the wavelength such that $\lambda f=c=3x10^{10}=$ constant (velocity of light). Figure-4 depicts multi-peak splitting with decreased MRF-resistivity trends between R= 275kΩ-0 with radioactivity exposure due to α-radioactivity radionuclide $_{95}Am^{241}$ ($_5f^4,_7s^2$), the exponential decay occurs after the last peaks. For H=2kG, three sharp peaks at f=0.6,3,5.5MHz with magnitudes Rmax=270,275,225kΩ, for H=4kG 3-sharp peaks at f=0.6,3,5.5MHz having Rmax=150,180,100kΩ, H=6kG, 2-sharp peaks at f=0.75,5.1MHz; Rmax=120,170 kΩ, for H=10kG, 3-sharp peaks at f=0.5,3,5.5MHz having Rmax=150,180,100kΩ respectively are observable. The minimum values of resistance at f=7MHz i.e. Rmin= 250kΩ at H=2,10kG and Rmin=500kΩ at H=4kG and Rmin~0Ω at H=6kG which is equivalent to superconducting phase transition in medicinal plant Alloy Veera.

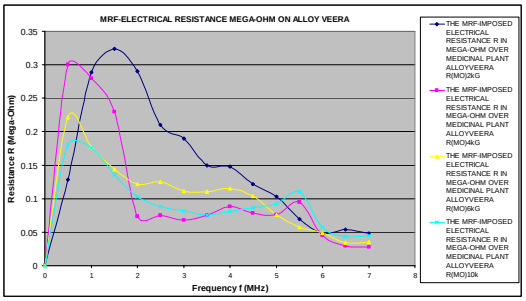

FIGURE- 3

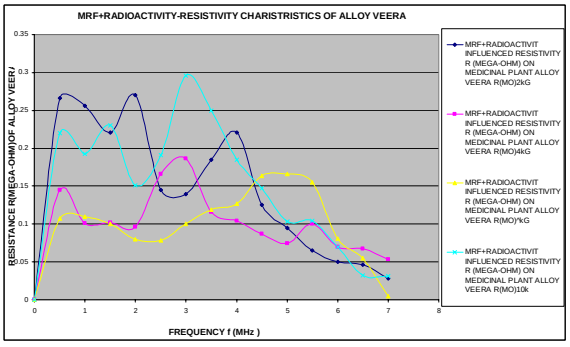

FIGURE- 4

Figure-5 shows electrical power transmission < 300 mega Watt received through the electrodes having radius r=0.075cm inserted

in *Alloy Veera*. For H=2kG, one sharp peak having maximum power dissipation Pmax=170μW at f=4.6MHz. For H=4kG a sharp peak Pmax=258μW at f= 3.6MHz, H=6kG, two sharp peaks having Pmax= 175,186μW at f=3.75,5MHz and one sharp peak for H=10kG, with Pmax=252μW at f=4.2MHz are transmitted. For Radioactivity stimulated MRF-Power Transmission as depicted in figure-6 there exist 13 sharp peaks for H=2kG, Pmax=40,84,102μW at f=0.5,3,4.6MHz, H=4kG, Pmax = 81, 63, 126μW, H=6kG, Pmax=76,109,137μW at f=0.6,2.1,4.1MHz, H=10kG, Pmax=49, 63, 88, 44μW at f=1.1,2.1,4.75,6.5MHz respectively. Radioactivity excitation suppresses MRF-Power transmission in *Alloy Veera* which is evident from a look at figures 5 and 6. One may easily compute that on utilization of one cm square area of leaf of *Alloy Veera* a power of ~0.014153 Watt may be obtained in the form of direct electrical energy whose input may be either from sun or earth or by both. Figure-7 represents the magnetic field dependence of longitudinal Ohmic voltage derivative of transverse MRF-potentials i.e. (dVH/dVx)-H Characteristic curves. All of these curves at frequencies f=2,3,4MHz are oscillatory between the magnitudes +(0.35) and –(0.15) each having double peak distribution.

Figures (8) and (9) are depicting the magnetic field H dependence of frequency derivative magneto-potentials i.e. (dVH/df)-H records at Vx=2 & 4 Volts respectively. The oscillatory behaviors of both of these curves indicate that the medicinal as well as resource exploration from earth can be externally regulated by employing optimum values of magnetic field H and the radio-frequency f-transmission processes through the medicinal plants like *Alloy Veera* in technologically driven in desired gross way. Such processes could also be activated in almost all the plant species but their resource generation capability remain restricted only up to a limit as they are not that resistant to withstand that outstanding limit which is exceptionally gifted by nature to the medicinal plants capable of sustaining their survival even in the hardest crisis of environment.

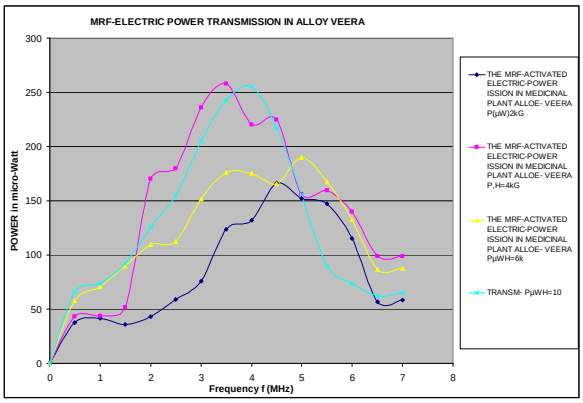

FIGURE- 5

The Radioactivity-MRF-Stimulations on medicinal plants sensitize them to develop vital capability to absorb more organic matter in fluid mixed shape and retain it's food entities in organic as well as inorganic shapes. At the same time this sensitization also enhances the Photo-Electric-Effect transforming food synthesis in the carbohydrate shapes absorbing CO_2, H_2O etc. from the environment under light radiation from sun. This is because the absorption band width and fluorescence band widths are broadened by MRF-stimulation just like magneto-optic switching, which is resulting in photo-synthesis at many more wavelengths rather than only those restricted ones which are entrusted by nature. Thus a grown up bio-synthesis resulting in biological growths and curing of creatures under artificially feeder MRF-signaling will emerge in big desired way. The metallic and other geo-resource entities associated with such sensitized bio-

matters will be proportionally large with their grown up bio-mass. The naturally occurring food flow in fluid state through capillary tubes and osmotic pressure conditions is also grown up because the porosity fluctuation due to electrical conductivity variation under MRF-perturbation increase porosity radii of capillaries along with large growth in osmotic pressure resulting in grown up bio-synthesis accumulating more geo-resources and transferring to the animal world. The computation of half widths $(\Delta\omega)_{1/2}$ of the resonance bands and their peak values obtained in the MRF-Magneto-potential records with charge-mass ratio of electrical carriers ensures the mobility of ionic transportation of Pb, Am, I,Xe,Mo,Hg,As, Te, Ag, Kr,Co,Ti,Cu,Cl,Na,Li,K,Au,Be,Mg,Ca, Cd,Al, etc. & some of them are the trace elements of organs maintaining balance in animal anatomy. An appropriate electrode-net-working may accumulate the ionic entities due to MRF-Stimulated Osmosis resulting in Plant-Electrolysis.

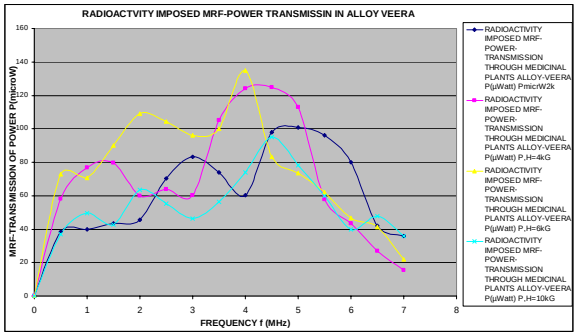

FIGURE- 6

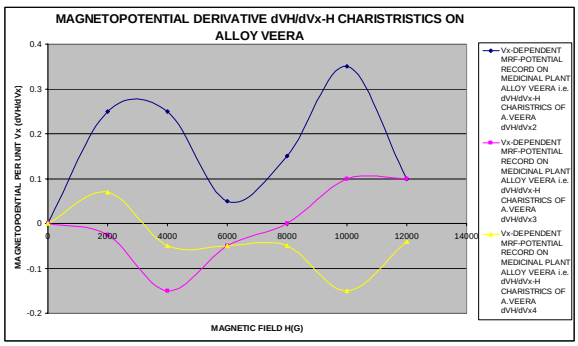

FIGURE-7

The various values of parameters like Hall coefficients R_H, electrical carrier concentration(n_H), mobility (μ), cyclotron frequency(ω_p) and free space wavelengths (λ_p) have been computed at various frequencies and their minimum values at f=2MHz; $R_H=0.0462\times10^{-15}$, $n_H=150.3\times10^{13}$, $\mu=0.675\times10^{5}$, $\omega_p=218.62\times10^{10}$ and $\lambda_p=0.0862$ cm and the maximum values at f=4.1MHz; $R_H=0.314\times10^{-15}$, $n_H=22.11\times10^{13}$, $\mu=6.29\times10^{5}$, $\omega_p=83.85\times10^{10}$ and $\lambda_p=0.2247$cm which are sufficiently large to be channelized using the appropriate networking on the various components of plant systems.

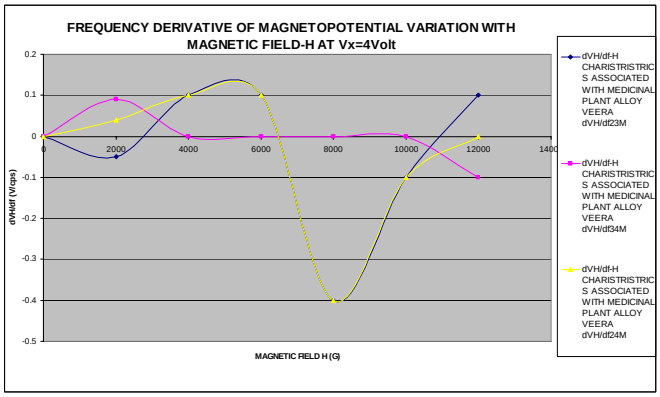

FIGURE- 8

Acknowledgement

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Results discussion & conclusion

The MRF-stimulated non-linear dynamic magneto-transport along with the tuber yield and nutrition quality (P & K) may be obtained on medicinal plants just as in potato (*Solanum L.*) grown under dual combination of chemical fertilizers and coal smoke, the pollutants[10].The multiple coaxial nano-layerings due to Radioactivity-MRF-Excitations in medicinal plants[12] may be utilized in drug delivery to equip the living race with potential technological tool to conquer dreaded diseases like cancer, AIDS etc. The characteristic plots of second derivative of trans-conductance in medicinal plants revealing peak-structures confirm their super-lattice configuration ensuring nano generations under MRF-Radioactivity perturbations. These artificially created nano-structures could serve as the external side support to bio-net-work facilitating the efficient functioning of bio-systems. The MRF-switching on them can tunnel the desired metals (as trace elements in different organs) and the other organic entities needed to maintain sound metabolism and excretion processes, directly and indirectly the endocryonological and neuro-signallings as bio-communication channel-network. At the same time, MRF-filtering of ionic and other organic fluid entities could be made externally to explore metals through co-axial nano-tunnels surrounding central macro-tunnel capable to flow fluid from earth to external environment vitally supporting the capillary systems associated with the bio-world. Such realities had been confirmed by the computation of porosity relationships with the electrical conductivity supported by the experimental evidences due to MRF-excitations in medicinal plants. Based upon MRF-stimulated mobile ions, applications may vary widely as follows;

Li^+, Na^+, Cu^+, Ag^+ Secondary Bio-Batteries

H^+ Bio-Fuel Cells, Secondary Bio-Batteries

O^{2-} Bio-Sensors, High Temperature Electrolysis

F^- Bio-Sensors

Thus, the beam of such mobile ions in plants may also be employed for the desired metal sythesis just as an alternate to achieve the naturally occurring role of Cosmic ray's like stone-metallic transformations etc. recently endeavored by modern Accelerators[16] using various nuclear reactions.

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Biology and growth dynamics of a hillstream catfish P*seudecheneis sulcatus* (Mc Clelland) from Uttarakhand, India

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Abstract

The paper deals with certain specific biological aspects of the fish *Pseudecheneis sulcatus* (Mc Clelland) collected from the river Alaknanda of Garhwal Himalaya in a 65 Km stretch between Karnaprayag and Srinagar Garhwal. Various morphometric characters of the fish are studied in relation to its total length. Length weight relationship and relative condition factor is studied to explore proper growth and well being of the fish. Breeding biology, maturation and fecundity was studied in order to gather information which will be helpful in its conservation and intensive culture. The natural food of fish was analysed by scientific study of its alimentary canal and the growth dynamics of fish was studied based on length frequency distribution and counting of growth rings on trunk vertebrae.

Keywords: *Morphometrics* | *Food* | *Breeding biology* | *Growth dynamics* | *P. sulcatus*

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Introduction

The knowledge of fishery biology is quite essential for efficient management of fishery resources. These studies include the racial analysis of the fish population, their length-weight relationship, relative condition factor, feeding and breeding habits, fecundity, age and growth and various other factors of population dynamics. The present study is an attempt to describe some important aspects of fishery biology of a coldwater fish *Pseudecheneis sulcatus* inhabiting the river Alaknanda in Garhwal Himalaya.

The Morphometrics plays an important role in the identification of species. The length-weight relationship can differ if variations occur within a population, so its study is also important in the fishery biology and fishery management besides its major utility in converting the length into weight and vice-versa. The investigation pertaining to the relative condition factor is equally important as it points out the robustness and well being of a fish. Food is the prime need of life hence the study of food and feeding habits becomes highly significant in fishery biology. The food preference, feeding habits, availability of food in the environment and the intensity with which fish feed is an important aspect to study. Breeding is an important aspect through which a living being maintains its race. In case of fish it is highly essential to know about exact time of maturity, frequency of spawning and number of

eggs that are likely to be spawned by the fish for fish culture and proper exploitation in the fish farm management. The knowledge of spawning periodicity gives information about its frequency in a year for a particular fish species. The importance of fecundity is obvious as it gives an idea about the number of eggs to be laid. Thus, if fecundity of a fish is known, it is easier to make arrangements for successful hatching of the eggs in a fish farm.

Ageing a fish is equally important as it gives an idea of its harvesting. In a commercial fishery, the knowledge of age and growth rate of a fish species is very important. After a particular age, the growth of fish is checked and thus there is no significant increase in the flesh. Some important researches in this field during recent years are accredited to Pathani (1981), Tandon and Johal (1983), Dobriyal and Singh (1987, 89, 93), Kapoor and Khanna (1994), Dutta Munshi and Ghosh (1994), Rautela (1999), Thapliyal (2002) and Bahuguna (2007).

Materials and Methods

The Fish *Pseudecheneis sulcatus* is a rare hill stream catfish. The specimens were collected regularly for two years from January, 2000 to December 2001 from the river Alaknanda in a stretch of about 65 km from Karnprayag to Srinagar Garhwal. In all 160 specimens were sampled during two year period of study. After taking all the measurement in fresh conditions, the fish were preserved in 5-8 % formalin for further study. For morphometric analysis, the characters were analysed in relation to total length of fish.

The equation for the length-weight relationship was computed by using the formula for general parabola $W = aL^n$ (LeCren, 1951) where W = weight of fish, L = length of fish and a and n are the constants. The linearity of regression was tested by the analysis of variance. Based on the data collected and computed for length-weight relationship, the Kn factor was calculated for different sexes monthwise and seasonwise to know the well being of the fish by the formula

$Kn = W / W^{--}$,

Where Kn =Relative condition factor W= observed weight and W^{--} = calculated weight.

The frequency of spawning and the spawning season were studied by tabulation of percentage occurrence of fish in various stages of maturity monthwise and sizewise. Thirty mature specimens were examined for fecundity analysis, collected during the spawning and pre spawning season in the years 2000 and 2001. The gut contents of fish were removed and preserved in 4 % formalin solution. The food was analysed both quantitatively as well as qualitatively by the point method (Hynes, 1950). Ageing biology was studied by two methods, the Length frequency distribution and the vertebral method. Growth characteristics were calculated after Tandon and Johal (1996).

Observations and Discussion

Morphometrics

During the course of present study, the catfish *Pseudecheneis sulcatus* (Mc Clelland) was observed to attain a maximum total length of 201 mm. Fish less than 90 mm could not be observed in the entire sample. The fish is a beautiful example of the hillstream adaptation. It has thick and stouter lips, a well developed adhesive disc on chest which has

up to fifteen transverse septa of overlapping skin and big and flatter pectoral and pelvic fins. The dorsal profile of the fish is convex as compared to the ventral. The mouth is ventrally placed which confirm the bottom feeding and carnivore nature of fish. The eyes are situated well behind the middle of the head. Barbels are also very short and stouter. Summarised data on ten different body measurements are presented in the Table 1. The fish were devided into 12 different length groups with a class interval of 1 Cm starting from a group of 9.0 – 9.9 cm to 20.0 – 20.9 cm. The first group has only one frequency whereas the group 12.0 – 12.9 cm has maximum 29 frequency. No fish was available in the size group 19.0 – 19.0 cm. In the present investigations on *Pseudecheneis sulcatus* it was observed that all the body parts grow in accordance with the total length of the body. The ratio of Head length: snout length (1.19 ± 0.181 to 1.38 ± 0.136) clearly indicates that the eye is situated well behind the middle of the head length. Second important observation is that the body depth is slightly more than head length (head length is 0.764 ± 0.082 to 0.897 ± 0.204 to maximum body depth).Third observation is that the dorsal fin (D1) is originated little before the pelvic fin. The fish also has an adipose dorsal fin situated in between pelvic fin and the anal fin.

Length-weight relationship and Condition factor

Length-weight relationship after regression analysis for different sexes, different seasons and pooled data is presented in Table 2. The samples collection throughout the year when grouped for different seasons showed a close relationship between their length and weight. The regression coefficient for sex wise and pooled data varied from a minimum of 5.54 for female (r = 0.772) to a maximum of 5.9 for the males (r = 0.865). For season and sex wise it was ranged from 2.95 (r = 0.619) during autumn to 6.5 (r = 0,869) during winter for males and from 3.5 (r = 0.68 in monsoon to 8.3 (r = 0.768) in summer for the female fish.

The value of relative condition factor (Kn) was calculated for each fish and finally the average Kn value for different sexes during each month was calculated and presented in Table 3. It shows that the relative condition factor was maximum 1.04 $\pm$ 0.16 during August in males and 1.0402 $\pm$ 0.33 during July in females. The lowest value 0.996 $\pm$ 1.7 for males and 0.9544 $\pm$ 0.643 for the females were observed in the month of October. The Kn values were also constant during November to march for both the sexes which is an indicative of stable substratum and availability of food in nature. Similarly the average Kn valves for male and female fish for different seasons were also calculated. The values were highest for both the sexes during monsoon (1.011 ± 0.1546 for the males and 1.0262 ± 0.267 for the females) due to sexual maturity. The values were also quite high during winter which again indicates the suitability of environment for food availability. The analysis of variance (ANOVA) between length and weight relationship for *Pseudecheneis sulcatus* (McClelland) sexwise and for the pooled data were observed insignificant (male – $F_{0.05}$ = 3.84, ddf = 154; female - $F_{0.05}$ $F_{0.05}$ = 3.84, ddf = 136; pooled date - $F_{0.05}$ = 3.84, ddf = 292). The analysis of variance for sex and season wise in *P. sulcatus* showed either insignificant or low significant values ($F_{0.05}$ ranging from 3.92 to 4.75), which showed positive relationship and also almost similar

growth in all the season. During monsoon the feeding was low but maturity in fish raised the value of relative condition factor. The values of the coefficient of condition or condition factor have been used widely by fishery investigators to express the relative robustness of fishes. In the present investigation Relative condition factor was calculated for *P. sulcatus*. Le Cren (1951) maintained that the condition factor is affected by length as well as several other factors like environment, food supply and degree of parasitism. As it makes its interpretation difficult, he suggested that the effect of length and its correlated factors may be eliminated by using a relative condition factor (Kn) which is based on the empirical (observed) and calculated length-weight relationship. In his work on perch *Perca fluviatilis* he indicated that Kn was function of fatness and condition of gonads. He also observed that there is a regular seasonal cycle in the relative condition, which is at its peak in September and minimum in early spring. The different seasonal changes in condition between mature and immature fish can largely be accounted for by the cycle in gonad weight of the former. Thus Kn is a superior measure than the K factor and is used to assess the effect of all sort of variations on the species.

Breeding biology

P. sulcatus is a rare hillstream catfish whose 50 % catch of the entire year was made during July- August when it is available in considerable quantity. The frequency distribution of ova diameter measurements from a mature ovary showed that a continuous growth of ova with a mode at 95 omd in a range of 55 – 95 omd. It seems that the fish spawned in more than one attempt from July – August. A few fish were also observed spawning in September. Study confirms a single frequency of spawning in the fish. The data pertaining to the occurrence of fish in different stages of maturity are presented in Table 4. Fish of advance maturity (stage VI) were observed during June – August and the spent fish during July-August. Only one spent fish was observed in the month of September. On the basis of above observations it can be inferred that the spawning in *Pseudecheneis sulcatus* takes place during July - August. Pionering work on the spawning behaviour in fish have been studied by Clark (1934) and Hickling and Rutenberg (1936) based on the size distribution of intra-ovarian eggs in different fishes. A majority of teleost fishes all over the world are seasonal breeder and in the Indian subcontinent a vast majority of freshwater fishes breed during the monsoon months of heavy rainfall (Jhingran, 1982). Present study indicated that *Pseudecheneis sulcatus* spawns for a limited period of July – August. According to Dobriyal et.al. (2000) *Barilius barna* spawns in the side waters of the stream Khandagad, a tributary of the Alaknanda river system. They lay their eggs in protected turbid shallow waters under stones. *P.sulcatus* also spawns in the flooded riverine conditions during monsoon season.

Absolute fecundity of the fish ranged from minimum of 1945 ± 692 in the fish measuring 127.4 ± 3.05 mm with body weight 15.08 ± 3.43 g, ovary length 36.8 ± 7.53 mm and ovary weight 1.6 ± 0.93 g to a maximum of 6435 in a fish of 200 mm body length, 54.2 g body weight, 49 mm ovary length and 5.23 g ovary weight (Table 5). A straight line relationship is obtained between fecundity and body parameters which was much closer in Fish weight - fecundity (r = 0.975) and ovary weight- fecundity (r =

0.965). Similar observations have also been reported by Pathni (1981), Agarwal *et.al.* (1988) and Dobriyal *et.al*. (1990). The sex ratio analysis has been considered of immense importance in the fisheries investigations. In the fish under study, the sex ratio was observed to be very close to the nature (1.05 male : 1 female).

Length group (Cm)	Standard length	Head length	M.B.D.	Snout length	Eye diameter	Pre-dorsal length	Pre-pelvicl length	Pre-anal length	CL
9- 9.9	1.225 (±0)	7.5 (±0.0)	6.53 (±0.0)	9.8 (±0.0)	98 (±0.0)	3.26 (±0.0)	3.16 (±0.0)	1.78 (±0.0)	6.53 (±0.0)
10- 10.9	1.27 (±0.066)	6.97 (±0.369)	6.23 (±0.913)	9.62 (±1.36)	69.2 (±28.43)	3.52 (±0.21)	3.13 (±0.046)	1.87 (±0.049)	4.79 (±0.865)
11- 11.9	1.24 (±0.022)	7.63 (±2.36)	6.50 (±0.869)	9.61 (±0.92)	62.8 (16.88)	3.41 (±0.258)	3.00 (±0.686)	1.88 (±0)	5.13 (±0.397)
12- 12.9	1.23 (±0.017)	6.70 (±0.650)	6.21 (±0.687)	9.22 (±1.203)	54.10 (±1.424)	3.42 (±0.185)	3.07 (±0.210)	1.94 (±0.304)	5.31 (±2.148)
13- 13.9	1.23 (±2.57)	7.44 (±0.569)	6.28 (±0.406)	8.84 (±1.34)	64.12 (±4.643)	3.34 (±0.641)	2.99 (±0.139)	1.95 (±0.079)	5.43 (±0.429)
14- 14.9	1.194 (±0.213)	7.48 (±0.919)	6.38 (±0.830)	10.68 (±1.571)	48.09 (±17.91)	3.35 (±0.251)	3.07 (±0.250)	1.92 (±0.208)	4.19 (±0.467)
15- 15.9	1.141 (±0.315)	6.81 (±0.701)	6.27 (±0.918)	8.91 (±0.997)	64.94 (±13.19)	3.30 (±0.829)	2.92 (±0.182)	1.84 (±0.0724)	5.57 (±0.54)
16- 16.9	1.21 (±0.014)	6.93 (±0.582)	6.07 (±0.675)	8.41 (±0.652)	65.21 (±13.94)	3.01 (±0.974)	3.29 (±1.128)	1.84 (±0.119)	5.27 (±1.679)
17- 17.9	1.16 (±0.207)	7.28 (±1.033)	6.58 (±1.69)	9.44 (±2.69)	67.6 (±14.69)	3.23 (±0.218)	3.0 (±0.16)	1.83 (±0.063)	6.19 (±0.576)
18- 18.9	1.20 (±0.0153)	6.99 (±0.483)	6.42 (±0.510)	8.24 (±0.31)	60.3 (±0.577)	3.25 (±.0346)	2.85 (±0.138)	1.86 (±0.0750)	4.07 (±0.436)
19- 19.9	-	-	-	-	-	-	-	-	-
20- 20.9	1.176 (±0)	6.45 (±0)	6.89 (±0)	8.33 (±0)	66.7 (±0)	3.33 (±0)	2.98 (±0)	1.90 (±0)	6.66 (±0)

Table 1: Body parts in ratio of total length in *P. sulcatus*.

S.N.	condition	parabolic equation	correlation coefficient (r)
1.	**Sex wise and pooled data.**		
	Male	$W = -51.67\ L^{5.9}$	0.865
	Female	$W = -50.43\ L^{5.54}$	0.772
	Pooled data	$W = -52.25\ L^{5.82}$	0.834
2.	**Season and Sex wise**		
	Male		
	Winter	$w = -60.89\ L^{6.5}$	0.869
	Summer	$w = -10.98\ L^{3.756}$	0.549
	Monsoon	$w = -41.03\ L^{4.88}$	0.951
	Autumn	$w = -7.487\ L^{2.95}$	0.619
	Female		
	Winter	$w = -65.56\ L^{6.54}$	0.912
	Summer	$w = -84.88\ L^{8.3}$	0.768
	Monsoon	$w = -24.95\ L^{3.5}$	0.686
	Autumn	$w = -35.39\ L^{4.42}$	0.683

Table 2: Regression analysis and coefficient of correlation on length- weight relationship of *Pseudecheneis sulcatus* based on data collected from Jan.2000 to December, 2001

Season	Relative condition factor (Kn.)	
	Male	Female
Winter	1.00128 ± 0.146	1.007 ± 0.1594
Summer	0.9991 ± 0.054	1.00026 ± 0.1507
Monsoon	1.011 ± 0.1546	1.0262 ± 0.267
Autumn	0.85392 ± 0.21402	0.989 ± 0.043

Table- 3: Seasonal fluctuation in Relative condition factor (Kn) for different sexes in *Pseudecheneis sulcatus* during January 2000- December, 2001

Months (1994)	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	No. of fish examined
Jan.	-	50.0	50.0	-	-	-	-	4
Feb.	-	-	66.7	33.3	-	-	-	3
Mar.	-	-	80.0	20.0	-	-	-	5
Apr.	-	-	-	75.0	25.0	-	-	4
May	-	-	-	66.7	33.3	-	-	3
Jun.	-	-	-	20.0	20.0	40.0	-	5
Jul.	-	-	-	5.8	29.5	35.2	29.5	17
Aug.	-	-	-	-	17.4	26.1	56.5	23
Sep.	80.0	-	-	-	-	-	20.0	5
Oct.	66.7	33.3	-	-	-	-	-	3
Nov.	50.0	50.0	-	-	-	-	-	2
Dec.	25.0	50.0	25.0	-	-	-	-	4

Table 4: Percentage occurrence of different maturity stages of *P. sulcatus* during January, 2000 to December, 20001

Fish length (cm)	Fish weight (gm)	Ovary length (cm)	Ovary weight (gm)	Fecundity
12.2-12.9 (12.74 ± 0.31)	11.0-18.52 (15.08 ±3.43)	3.0-4.8 3.68 ±0.753	0.715-4.9 1.60 ± 0.93	1299-2842 1945 ± 692
13.0-13.9 (13.53 ± 0.35)	11.9-33.31 (21.82 ±5.77)	3.2-4.8 3.69 ±0.46	0.98-2.89 1.587 ± 0.617	1490-2491 2073 ± 412
14.0-14.6 (14.4 ± 0.23)	24.1-34.4 (29.09 ±4.17)	3.3-4.7 3.94 ±0.58	1.3 - 3.12 2.149 ± 0.819	1988-6212 4058 ± 1947
15.0-15.9 (15.38 ± 0.38)	31.0-42.9 (36.46 ±4.01)	3.3-4.8 4.21 ±0.53	1.75-4.75 3.32 ± 0.93	2633-6021 4873 ± 1181
16.2-16.4 (16.3 ± 0. 14)	40.82-44.7 (42.75 ±2.74)	3.6-5.1 4.35 ±0. 35	4.115 -4.82 4.47 ± 0.49	5411-5885 5648 ± 335
17.0 ± 0.0	46.2 ±0.0	4.1 ±0.0	4.8 ±0.0	6099 ± 0
18.0 ± 0.0	52.72 ±0.0	4.6 ±0.0	4.9 ±0.0	6266 ± 0
20.0 ± 0.0	54.2 ±0.0	4.9 ±0.0	5.23 ±0.0	6435 ± 0

Table 5: Data on the Reproductive capacity of *P. sulcatus* collected from river Alaknanda in a 65 km stretch from Karnprayag to Srinagar during spawning season (2000 - 20001)

SEASONS	DIFFERENT FOOD ITEMS AND OTHER CONTENTS PRESENT IN GUT				
	Insects	Flesh	Green matter	Diatoms	Sand and debris
Summer (March-June)	78.905±7.190	2.934 ± 4.424	7.65 ± 4.99	3.64±1.410	3.39 ±4.04
Rainy (July-August)	41.77± 6.366	33.91 ± 1.65	6.92 ± 1.1377	1.525 ± 0.403	15.97±3.79
Autum (Sep- Nov)	84.46±4.65	3.92±2.24	4.55±2.75	4.22 ±0.25	2.75 ±1.29
Winter (Dec-Feb)	77.96±9.38	9.339 ± 11.49	3.55 ±1.175	2.456 ±1.586	6.67 ±9.228

Table 6: Seasonal fluctuation in percental value of the gut contents of *P.Sulactus*

Parameters	Years of life (Age Classes)			
	1	2	3	4
L (mm)	89.8	120.7	139.5	143.0
h (mm)	89.8	30.9	18.8	3.5
Φh (mm)		46.56		
C_l		30.2	20.24	2.51
C_{lt}		0.396	0.276	0.0037
		0.225		
C_{th}		23.94	21.77	0.346
		15.852		

Table 7: Growth Dynamics *Pseudecheneis sulcatus* collect from river Alaknanda during 20(2001

L = Average length at the time of annulus formation, h = Annual growth increment, Φh = Index of species average size, C_l = Specific rate of linear growth, C_{lt} = Growth constant, Cth = Growth characteristics

Feeding biology

The quantitative analysis of food items were made by the point and percentage method. Monthly variation in percental values of various food items found in the gut of Pseudecheneis sulcatus (Mc Clelland) are presented in Table 6. Food analysis indicated that the insects were the main food of the fish. Animal flesh and earthworms were also found in abundance during monsoon season. Green algae along with diatoms were also observed throughout the year in small quantity. Thus the fish is classified as carni- omnivore and bottom feeder.The insect food dominated the entire gut contents and were minimum 37.29 ± 38.95 % during July and 88.33 ± 7.64 during April. Animal flesh, mostly earthwaorms, was maximum in July (35.08 ± 39.72 %) and nil in the month of April. Green algae and the diatoms were available throughout the year in small quantity. They were maximum in May (14.67 ±± 21.94 and 5.0 ±0.0 % respectively.) and minimum in the months of October (green algae – 1.75 ± 1.25 %) and July (diatoms (1.24 ± 1.49 %). The gut was full of sand and debris during July- August.

Most favourable season of feeding for *P. sulcatus* was observed as winter when the growth of insects and also that of algae is at its peak. Also, the environmental conditions are better and there is a minimum human interference. The velocity of water current is lowest and due to transparency, growth of algae and penetration of light rays upto the bottom, the primary as well as the secondary productivity is fine. During monsoon season the feeding is adversely affected due to disturbed environmental conditions. The GSI vale is high during monsoon due to the fact that the animal flesh was observed in the gut as this particular species is better caught by the fishermen with the help of angling using earthworm or flesh as bait. The rest of the food is rare. Another important reason might be that the fish is mature in this period and hence does'nt prefers feeding.

Age and Growth Dynamics

The vertebrae of *P. sulcatus* were found suitable for age and growth studies. The opaque and hyaline zones were distinct hence the ring which is all around the diameter was considered as an annuli. After using a standard regression equation, a straight line relationship was obtained for fish length and vertebrae diameter (VD = 4.116 + 8.4147 FL). In the present study 4 age rings were counted. The probable months of ring formation were adjudged as July- August. When the back calculation method was used, it was observed that the first, second, thrd and fourth ring was formed at an average length of 89.8 mm, 120.7 mm, 139.5 mm and 143 mm respectively. The length frequency distribution of 148 specimens collected round the year showed an annual bunching at the lengths 100 mm, 120mm, 140 mm and 150 mm respectively. The absolute length increment (h) was observed to be 89.8, 30.9, 18.8 and 3.5 mm respectively during first, second, third and fourth year respectively (Table 7). The specific rate of linear growth (C_l) was observed to be 30.2 between 1st and 2nd year, 20.24 between 2nd and 3rd year, and 2.51 between 3rd and 4th year respectively. The index of species average size (Φh) was calculated as 46.56 mm. The value of growth characteristics (C_l) showed higher growth (23.94) during 1st and 2nd year followed by 21.77 during 2nd and 3rd year. It was lowest during 3rd and fourth year (0.346).

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Comparison between organic and inorganic soil microbial diversity of different agronomic fields

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Abstract

Microorganisms are found in prominent amount with great species diversity in the soil of the earth. During the comparative study of organic and inorganic soil microorganisms the extent of the diversity of bacteria and fungi was enormous in organic soil comparatively to Inorganic soil. The development and application of methods to explore microorganism diversity in organic and Inorganic soil has revealed that a remarkable diversity of bacteria and fungi were found in organic soil as compared to Inorganic soil. The reason for this was that the organic soil nourished with organic matter (manures) provided an important habitat for bacteria to grow. The results so far showed that the abundance of different bacterial groups and total bacterial biomass was generally increased by organic matters in comparison to Inorganic fertilizers. It was found that with organic manures was rich in bacterial diversity (999 colonies) and eight genera comprising of 12 species of bacterial and fungal diversity (131 colonies) and species richness was higher in fungi as eighteen genera comprising of 39 species were found that the organic soil was highly diverse than Inorganic soil. Thus organic fertilizers have changed soil microorganism community structure and we propose the fact that the soil treated with organic fertilizers is the key factor determining that soil bacterial and fungal diversity is related to complexity of the microbial interactions in soil, including interactions between microorganisms and soil, and microorganisms and plants.

Keywords: *Organic | Soil Microbiology | Bacteria | Fungi | Agronomic Fields*

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Introduction

Soil represents a favorable habitat for microorganisms and is inhabitated by a wide range of microorganisms. Microorganisms are found in large numbers in soil usually between one to ten million microorganisms are present per gram of soil with a dominant number of bacteria and fungi. Soil organisms contributes to important soil functions such as supporting the growth of plants both in natural plant communities and those grown for food, fibre, or energy and absorbing, neutralizing and transforming compounds that might otherwise become pollutants in the environment. Soil microorganisms are very

important as almost every chemical transformation taking place in soil involves active contribution of these microbes. Soil microbial diversity is influenced by both organic and inorganic matter. Soil organic matter is generally used to represent the organic constituents in the soil, excluding undecayed plants and animal tissues, their partial decomposition products and the soil biomass (Dick, 1992). The soil organic matter provides a favorable habitat for the microorganisms to grow as compared to inorganic soil. The bacterial diversity present in the soil is greatly influenced by organic matter. It has been consistently reported that soil organic matter favors the growth of bacteria present in soil. The studies have revealed that bacterial diversity is approximately one hundred times greater than the other microbial diversity (Barns *et al.*, 1999).

Bacteria are one of the most important components of the soil micro biota and don't occur freely in the soil solutions but are closely embedded in organic matter even often adding as the dispersing agents (Atlas *et al.*, 1991). Moreover they play a major role in organic matter decomposition, biotransformation, biogas production, nitrogen fixation. In particular they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like potassium, phosphorous and nitrogen which are required for plant growth (El Frantroussi *et al.*, 1999).

In most of the aerated or cultivated soils fungi share a major part of the total microbial biomass. Many important plant pathogens and plant growth promoting microorganisms are fungi. Fungi are also critical decomposers in soil habitat like bacteria; fungi derived nutrients for their growth from organic matter (Bossio *et al.*, 1998). The rest being actinomycetes, protozoa, algae and many other also constitute the microbial diversity of soil. Microbial biomass in the soil display a positive linear relationship with annual net primary productivity, demonstrating that the growth of microorganisms and of crops can be controlled and influenced by using organic matter (Zak *et al.*, 1994).

Study site

For the comparison of microbial diversity in the organic and inorganic soil fields, the soil samples were taken from the "Biodiversity Conservation Farm" of NAVDANYA RESEARCH FOUNDATION run by an eminent environmentalist Dr. vandna shiva of India. This farm is located at Ramgarh about 16 km away from Dehradun. The Navdanya research foundation is working under the thought of organic farming and the methods for agriculture and post cropping activiyies are met with the global sustainable use of soil, organic fertilizers etc. Futhermore, the "*movement of protection of seeds*" is successfully running from the foundation, especially the grain seeds and pulses used in the hilly areas of Uttarakhand since time immemorial.

Materials and method

Isolation of bacteria

Isolation of microbes from soil took many steps which includes field trips, lab work etc. Soil samples were collected by sterile methods from organic and inorganic Sample plots/fields visited during the time of fructification of crop and brought to the lab in an air tight polybags. The vertical samples were taken from 5 and 10 cm depth. The samples were processed using soil dilution plate method. One gram of soil sample was

serially diluted with sterilized distilled water upto 10^{-1}, 10^{-2}, $10^{-3,}$ 10^{-4}, 10^{-5} 10^{-6}, 10^{-7} and 100 ml of each dilution was added to 20ml of nutrient agar medium in 90mm diameter sterile Petri dishes and then enumerated. Single separate colonies on the agar plates were selected at random according to standard medium and streaked on the nutrient agar slants and incubated for 24 hrs at ± 30^0 C. Code names were given to each of the isolated plates and stored at ± 4^0 C for characterization and identification by standard methods. Once colonies rose in the media, the sub culturing was continued until a pure isolate was obtained. Identification of microbes was done with the help of standard literature. For isolation of bacteria different media like Nutrient agar medium, Nutrient broth medium etc. (Hi media) were prepared and to differentiate between gram + ive and gram – ive bacteria Gram's staining was done. Thereafter for identification different biochemical tests were performed for both organic and inorganic bacterial colonies (Grant and Holt, 1977).

Isolation of fungi

The soil borne fungi was isolated and their total population was enumerated by following the method as given below:-

First soil samples were collected from both the organic and inorganic fields, then 3 flasks (250 ml) were taken and 90 ml distilled water was transferred into each flask. Each flask was plugged properly, labeled 1-3 and autoclaved at 15lb/inch^2 for 30 minutes. 1 gm of soil sample was weighed and transferred into the flask 1containing 90 ml. It gives the dilution 1:10 i.e. 10^{-1}.Then it was shacked for five minutes gently with a stirrer to get homogenous soil suspension. 1.0 ml soil suspension was transferred from 10^{-1} dilution into flask 2 containing 90 ml distilled water to get dilution 10^{-2} and then mixed it gently. Similarly 1ml of soil suspension was serially transferred from 10^{-2} dilution into flask 3 containing 90 ml water to get the final dilution of 10^{-3} and mixed it gently. 1ml of soil suspension was aseptically poured from 10^{-3} dilution in different media plates. The plates were gently rotated so as to spread the suspension on medium. The plates were incubated at ± 25^0 C for 4-5 days. Different media like Czapek Dox Agar medium, Potato Agar medium, Martin's Rose Bengal medium, etc. were prepared for isolation of fungi. For identification of fungi lacto phenol and cotton blue stain was used also called as mounting fluid. The slides were observed under microscope and fungi were identified by following the mycological literature. (Dubey & Maheshwari, 2007).

Results and Discussions

An acceptable number of fungi in 1g of fertile soil are around 400000 (Griffith *et al.,* 1999). However in the present study a total of 1130 isolates of microbes comprising of bacteria, fungi, actinomycetes were obtained from the analysis of 8 soil samples taken four from organic and four from inorganic sample fields in September 2008. The serial dilution method was followed to determine the microbial diversity of soil. The identification of these isolates resulted in 51 species of microbes including bacteria (11 species), fungi (39 species) and actinomycetes (1 species). The genera with the greater number of species in fungi were *Aspergillus* (8 species), *Glomus* (4 species), *Penicillium* (3 species). *Cladosporium* (3 species) in the serial dilution plate method. The most widely distributed and abundant colony forming taxa were *Penicillium* (16 colonies), *Aspergillus* (29 colonies), *Rhizopus* (10 colonies)

Trichoderma (10 colonies),*Fusarium* (10 colonies), *Glomus* (8 colonies), *Cladosporium* (8 colonies) and *Humicola* (7 colonies) in both soil sample fields. The richest genera in terms of the number of species were *Aspergillus* and *Penicillium* and the most common ones in both sample fields were *Aspergillus niger, A. versicolor, A. flavus, A. candidus, Penicillium rubrum, P. puberlum, Cladosporium cladosporiodes, Trichoderma lignorum* and *Glomus mosseae*. Thirty nine fungal species representing 18 genera were isolated and identified from organic and inorganic fields. Three species belonged to genus *Penicillium* and *Cladosporium*, four to *Glomus a*nd eight to *Aspergillus*. Two species belonged to each genus of *Rhizopus, Mucor, Humicola, Fusarium, Gigaspora, Trichoderma* and *Scutellospora.* While the rest of the genera *Acremonium, Verticillium, Acaulospora, Albidia, Alternaria, Chrysosporium* and *Sclerocystis* were represented by a single species.

In bacteria the results showed that the number of colonies was found higher in organic field (677 colonies) in comparison to inorganic field (322 colonies). The study also showed that bacterial colonies grew in potato dextrose and malt extract medium were higher than fungal colonies which proved the earlier research that bacteria produce different kinds of enzymes which inhibit the other fungal species in soil whether that are useful or pathogenic to the crops. Pseudomonas and bacillus genera were found both in organic and inorganic fields and were proved higher in species richness among other bacterial species. This data proved that both the bacterial genera were able to tolerate adverse microclimate in soil and decompose organic material and can synthesize inorganic minerals. Moreover they can sporulate properly and helped in making soil much nutritive with the help of different types of enzymes produced by them (Westower *et al.*, 1997). On the other hand *Clostridium Perfringens, Streptomyces, Flavobacterium sp., Azotobacter vinelandii, Azospirillum* sp. and *Escherchi coli* were not isolated from inorganic soil samples may be due to their non occurrence in these types of soils or could not rose in used culture medium.

The isolation of various fungal, bacterial species showed that the soil of organic field is quite rich in microbial flora. A total of 93 colonies of fungi and 677 colonies of bacteria were isolated from organic field, while a total of 38 colonies of fungi and 322 colonies of bacteria were isolated from inorganic field.

The results showed that soil was rich in bacterial diversity (999 colonies) comparatively to fungal diversity (131 colonies), however species richness were high in fungi as eighteen genera comprising of 39 species were found in both types of fields, while only 8 genera comprising of 12 bacterial species has been found. Since the soil samples were taken during fruiting time of crop, at the fruiting time exchange of mineral production of different micronutrient material by bacteria was so high, thus many colonies were found on triplicate agar plates. The organic field nourished with cow dung, ashes, mulches, vermicompost etc. produced high number of bacteria and fungi comparatively to inorganic fields where only 9 species of bacteria and 21 species of fungi were recorded which is nourished with chemical fertilizers. Among 39 species of fungi, 29 species were isolated from both organic and inorganic fields while 18 species could not found in inorganic field. The occurrence of plenty of species in genus *Aspergillus* (8) and *Penicillium* (3) are

probably due to their capability of producing a diverse range of antibiotics and mycotoxins which protect them from other soil organisms and may also hinder the growth of other fungal species. The percent occurrence of these fungal species is given in the table 2. *Aspergillus niger* and *Penicillium rubrum* showed occurrence frequency of 3.8% in Organic field and 3.1% in Inorganic field. *A. nidulens, cladosporium cladosporoides, Fusarium oxysporium, Mucor mucido, Rhizopus arrhizus, Trichoderma lignorum*, showed 3.1% occurrence frequency in organic field, while in Inorganic field the percent occurrence is 2.3%, 0.8%, 1.5%, 3.1%, 1.5%, 1.5% respectively for above species. Whereas the lowest occurrence frequency was shown by *A. flavus, A. candidus, A. ustus, Cladosporium, C. elatum, Gigaspora remisporophora, Glomus fasciculatum, Mucor heimalis, Sclerocystis gregarea and Verticillum candelabrum* as there were isolated not more than 0.8% of total colonies of fungi. Some other species i.e, *Absidia ramosa, Acaulospora elegans, Acremonium stomaticum, Aspergillus versicolor, A. terreus, A. fumiculosus, Alternaria brassicola, Cladosporium lunata, Gigaspora gigantea, Glomus mosseae, G. formosanum, Humicola grisea, H.fusco-atra, Penicillium puberrulum, P. purprogenum and Trichoderma virde* have showed moderate percentage of occurrence from1.5% to 2.3% on agar plates. The same results were found with the bacteria where the percentage occurrence was higher in organic field comparatively to Inorganic fields.

These results showed that a positive correlation exists between percentage of occurrence and the number of soil samples from which fungal species were collected. The significance of this correlation is that within a specific area we can determine the distribution of a particular fungal species from its occurrence frequency (Yang and Crowley, 2000). The study extends to relate occurrence frequency with sporulation and growth rate on culture. Twenty one species were collected from both types of fields with much variation in microclimatic conditions. These include *Aspergillus flavus Aspergillus niger, Aspergillus versicolor, Cladosporium cladosporioides,Mucor hiemalis, Penicillium rubrum, P. purprogenum and Trichoderma lignorum.* Their isolation from both types of localities and especially those one with highest occurrence frequency showed that these are physiologically more versatile, not only in coping with the harshness of the microclimate but also have an extensive enzyme system to degrade organic matter of various nature (More, 1994). This study also proved that organic fertilizers have great capacity to give a good atmosphere for microbial growth comparatively than in Inorganic fertilizers because synthetic fertilizers depends on the chemical reactions while due to organic fertilizers, natural physiological activities occur among various microbes.

The consequence of the present study that the organic farm soils have a great capacity to give space to the microbial survival which renders a fruitful outcome in the form of good crop production having a great tolerance to atmospheric pathogens and diseases. At the same time inorganic soil has less microbial diversity which proved that some bacteria or fungal species may be found in inorganic fields but not able to use properly the microclimate or micronutrients as can be used by organic microbes. The mycorrhizal species i.e. *Gigaspora, Glomus, Sclerocystis, and Scutellospora* have been found in ample

amount in organic fields while only a single colony has been found in inorganic field. Furthermore the results indicate that common practices of using synthetic fertilizers harms the soil quality in time and consequently low fertility of soil can be observed.

Thus from the results we concluded that organic soil was rich in microbial diversity in comparison to Inorganic soil whether the microbes were bacteria, fungi, etc. It was because the soil with organic matter provides a favorable habitat for microbial diversity whereas inorganic soil with chemical fertilizers has harmful effect on the growth of microbial diversity and cannot survive.

			Organic field	Organic field	Inorganic field	Inorganic field
S.No.	**Field**	**Dilution**	**No. of colonies in serial Dilution method**	**No. of organisms per gram of soil**	**No. of colonies in serial Dilution method**	**No. of organisms per gram of soil**
1	A*	10^{-4} 10^{-5} 10^{-6} 10^{-7}	15 5 2 -	22×10^{-7}	7 3 1 -	11×10^{-7}
2	B*	10^{-4} 10^{-5} 10^{-6} 10^{-7}	12 8 6 -	26×10^{-7}	7 4 1 -	12×10^{-7}
3	C**	10^{-4} 10^{-5} 10^{-6} 10^{-7}	12 8 3 -	23×10^{-7}	5 2 1 -	8×10^{-7}
4	D**	10^{-4} 10^{-5} 10^{-6} 10^{-7}	14 6 2 -	22×10^{-7}	4 1 - -	5×10^{-7}
	Overall Total colonies		93		38	

Table 1: Occurrence of fungal colonies in serial dilution method in both organic and inorganic fields

S. No.	Fungi from organic field	Fungi from organic field	Fungi from organic field	Fungi from Inorganic field	Fungi from Inorganic field	Fungi from Inorganic field
	Species	No. of colonies	Occurrence %	Species	No. of colonies	Occurrence %
1	*Absidia ramosa*	2	1.5%	Not found	-	-
2	*Acaulospora elegans*	2	1.5%	Not found	-	-
3	*Acremonium stomaticum*	2	1.5%	Not found	-	-
4	*Alternatia brassicicola*	3	2.3%		2	1.5%
5	*Aspergillus niger*	5	3.8%		4	3.1%
6	*A. nidulens*	4	3.1%		3	2.3%
7	*A.terreus*	3	2.3%	Not found	-	-
8	*A.versicolor*	2	1.5%		1	0.8%
9	*A.candidus*	1	0.8%		1	0.8%
10	*A.flavus*	1	0.8%		1	0.8%
11	*A.fumiculosus*	2	1.5%	Not found	-	-
12	*A.ustus*	1	0.8%	Not found	-	-
13	*Chrysosporium sp.*	3	2.3%		1	0.8%
14	*Cladosporium cladosporoides*	4	3.1%		1	0.8%
15	*C.lunata*	2	1.5%	Not found	-	-
16	*C.elatum*	1	0.8%	Not found	-	-
17	*Fusarium oxysporium*	4	3.1%		1	0.8%
18	*F.moniliformae*	4	3.1%		2	1.5%
19	*Gigaspora gigentium*	2	1.5%	Not found	-	-
20	*G. remisporophora*	1	0.8%	Not found	-	-
21	*Glomus mosseae*	3	2.3%		1	0.8%
22	*G. fasciculatum*	1	0.8%	Not found	-	-
23	*G.ambisporum*	1	0.8%	Not found	-	-
24	*G.formosanum*	2	1.5%	Not found	-	-
25	*Humicola grisea*	3	2.3%		2	1.5%
26	*H. fusco-atra*	2	1.5%	Not found	-	-
27	*Mucor mucido*	4	3.1%		2	1.5%
28	*M. hiemallis*	1	0.8%		1	0.8%
29	*Penicillum rubrum*	5	3.8%		4	3.1%
30	*P. puberrulum*	3	2.3%		1	0.8%
31	*P. purprogenum*	3	2.3%	Not Found	-	-
32	*Rhizopus arrhizus*	4	3.1%		2	1.5%
33	*R. oryzae*	2	1.5%		2	1.5%
34	*Sclerocystis rustiformis*	1	0.8%	Not found	-	-
35	*Scutellospora pellucid*	2	1.5%	Not found	-	-
36	*S. gregarea*	1	0.8%	Not found	-	-
37	*Trichoderma lignorum*	4	3.1%		2	1.5%
38	*T. viride*	3	2.3%		1	0.8%
39	*Verticillum candelabrum*	1	0.8%	Not found	-	-
Total		93			37	

Table 2: Percentage of occurrence of various fungus species colonies in examined field areas

S.No.	Field	Dilution	Organic field No. of colonies in serial Dilution method	Organic field No. of organisms per gram of soil	Inorganic field No. of colonies in serial Dilution method	Inorganic field No. of organisms per gram of soil
1	A*	10^{-4} 10^{-5} 10^{-6} 10^{-7}	84 60 17 7	168×10^{-7}	45 43 9 1	98×10^{-7}
2	B*	10^{-4} 10^{-5} 10^{-6} 10^{-7}	77 49 17 6	149×10^{-7}	39 32 6 -	77×10^{-7}
3	C**	10^{-4} 10^{-5} 10^{-6} 10^{-7}	62 54 16 3	135×10^{-7}	43 32 2 -	77×10^{-7}
4	D**	10^{-4} 10^{-5} 10^{-6} 10^{-7}	55 26 17 4	102×10^{-7}	39 11 3 -	53×10^{-7}
	Overall Total colonies		677		322	

Table 3: Occurrence of Bacterial and actinomycetes colonies in serial dilution method in both organic and inorganic fields

*Wheat field; **Pea field in both organic and inorganic field

S. No.	Experimental Procedure	Observations and Results					
		C1	C2	C3	C4	C5	C6
1	Gram stain	+	+	-	-	-	-
2	Shape and arrangement	Rod shaped	Rod shaped	Thick Rod shaped	Thin Rod shaped	Thick Rod Shaped	Thick Rod shaped
3	Cultural Characteristics	Round creamy white	Transparent white	Round creamy white	Irregular creamy white	Round creamy white	Round creamy white
4	Carbohydrate fermentation						
	a)Glucose	+	+	+	+	+	+
	b)Sucrose	+	+	+	+	+	+
	c)Fructose	+	+	+	+	+	+
5	Starch hydrolysis	+	+	-	+	-	-
6	Catalase activity	-	+	-	+	-	+
7	Indole activity	+	-	-	+	-	-
8	Methyl Red test	+	+	+	-	+	+
9	Voger Proskauer test	-	+	-	+	+	-
10	Oxidase test	+	-	+	+	-	+
11	Citrate utilisation	-	+	-	-	+	+

Table 4: Showing the description of unknown samples isolated from organic field

+ive = Present, -ive = Absent

S. No.	Experimental Procedure	Observations and Results				
		C1	C2	C3	C4	C5
1	Gram stain	+	+	+	-	+
2	Shape and arrangement	Rod shaped in chains	Thin Rod chains	Thick Rod shaped	Rod shaped	Rod Shaped in chains
3	Cultural Characteristics	Round creamy white	Round creamy white	Irregular transparent	Light yellowish colony	Round creamy white
4	Carbohydrate fermentation					
	a) Glucose	+	+	+	+	+
	b) Sucrose	+	+	+	-	+
	C) Fructose	+	-	+	+	+
5	Starch hydrolysis	+	-	-	+	+
6	Catalase activity	-	+	+	+	-
7	Indole activity	+	+	-	+	-
8	Methyl Red test	-	-	+	+	+
9	Voger Proskauer test	+	+	+	-	-
10	Oxidase test	-	+	-	+	+
11	Citrate utilisation	+	+	+	-	+

+ive = Present, -ive = Absent

Table 5: Showing the description of unknown samples isolated from Inorganic field

S.No.	Bacteria from Organic field			Bacteria from Inorganic field		
	Species	No. of colonies	Occurrence %	Species	No. of colonies	Occurrence %
1	Pseudomonas florescens	115	20.8%		71	23.2%
2	Bacillus megaterium	99	17.9%		62	20.3%
3	Bacillus cocii	87	15.7%		41	13.4%
4	Clostridium perfringens	43	7.8%	Not found	-	-
5	Azotobacter vinelandii	78	14.1%		44	14.4%
6	Escherichia coli	37	6.7%	Not found	-	-
7	Flavobacterium	74	13.4%	Not found	-	-
8	Streptomyces	21	3.8%		11	16.3%
Total		677			229	

Table 6: Percentage of occurrence of various bacterial species in examined field area

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Physico-chemical characteristics of a fish pond near Roorkee

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Abstract

This paper deals with the physical and chemical characteristics of the water of a fish pond near Roorkee. The present study was carried out from the month of January 08 to December 08 (1 year). The physico-chemical parameters such temperature, turbidity, pH, conductivity, dissolve oxygen, COD, BOD, free CO_2, total solids, TDS, TSS, total hardness, Ca hardness, Mg hardness, alkalinity, acidity, and chlorides were analysed during the course of study. The minimum and maximum range of physico - chemical properties were as, temperature 18.15 - 32.47 °C in January and July respectively, turbidity 62.25 - 236.25 JTU in January and August respectively, conductivity 582.75 µmho/cm in February and 1164.25 µmho/cm in July, total solids were 327 mg/l in February and 792 mg/l in July, total dissolved solids were 290.75 - 581.5 mg/l in February and July, total suspended solids were 31.75 mg/l in January and 242.25 mg/l in August , pH 7.78 – 8.43 in July and March respectively, dissolve oxygen was 5.9 mg/l in August and 7.02 mg/l in February, BOD was 4.27 mg/l in October and 4.82 in March, COD 8.5 – 9.9 mg/l in November and April respectively, free CO_2 was 2.38 – 3.38 mg/l in September and March, Acidity was ranged between 7.75 – 8.72 mg/l in September and March respectively, alkalinity 229 – 311 mg/l in August and December respectively, hardness 193 mg/l in February and maximum 386 mg/l in July, calcium 25.32mg/l in February and 50.26 mg/l in July, magnesium 31.51 in February and 63.26 mg/l in July, and chloride was minimum 31.3 mg/l in January and maximum 42.27 mg/l in August.

Keywords: *Physical Parameters* | *Chemical parameters* | *Pond water quality*

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Introduction

Much of the current concern with regards to environmental quality is focused on water because of its importance in maintaining the human health and health of the ecosystem. The ever-growing demands for water resources coupled with the rate at which much of the earth's fresh waters are being adversely affected by human activities, demonstrates a developing crisis in the not-too-distant future if environmental water resources are not appropriately managed (Peter et al. 1997). It

is therefore obligatory to have most relevant information for arriving at conclusive rational decisions that will ensure sustainable development of all these resources. Accurate and reliable information on the water resource system can therefore be a vital aid to strategic management of the resources (Gupta and Deshpande, 2004). Physicochemical factors are very important in estimating the constituents of water and concentration of pollutant or contaminant. Water quality generally means the component of water which must be present for optimum growth of aquatic organisms. The determinant of good growth in water body includes dissolved oxygen, hardness, turbidity, alkalinity, nutrients, temperature, etc. Conversely, other parameters like biological oxygen demand, and chemical oxygen demand indicate pollution level of a given water body. Accurate assessment depends on the results generated by specific monitoring activities which define the physical, chemical and biological condition of the resource (Khanna *et al*. 2007). Ponds, Lakes and Reservoirs, as integral components of our planet's life support systems, are essential for the maintenance of human life. These exists a strong interdependence between human population and aquatic ecosystems. The monitoring of the pond water is an essential step to mark the trend pattern of pollutants and their effect on living systems in today's developing life.

Materials and Methods

Pond is situated 5 Km. away from Roorkee in village Paniyala. The area of the pond of 75 Bhiga.The source of water in pond is rain water. Pond is situated at height of 268 meter from the mean sea level (longitude 77 degree 53 minute East and latitude 29 degree 51 minute North). The depth of pond is 2.8 meter and minimum 0.6 meter, mean depth is 1.7 meter and surface area of the pond is 2,92,500 square meter , catchments area is 2,92,400 square meter. Analysis of water samples were done according to standard methods as prescribed in APHA (2005), Trivedi and Goel (1986), and Khanna and Bhutiani (2008). Monthly samples were collected from pond from different location sites. The samples collection was completed during morning hours between 7:00 A.M. To 09:30 A.M. Physico-chemical parameters such as temperature, dissolved oxygen, electrical conductivity and pH value were measured directly in the field. Temperature (by using precise mercury thermometer), hydrogen ion concentration (by using pH-meter), electrical conductivity (by using EC-meter), turbidity level (by using turbidity-meter), dissolved oxygen (titrimetric methods), biological and chemical oxygen demand (titrimetric methods),carbonate and bicarbonate were determined directly by titration with standard 0.02 N HSO using phenolphthalein and methyl orange as indicators. The total chloride was measured by titration of 50 ml of sample against silver nitrate (0.0141 N) solution using potassium chromate as indicator.

Results and Discussion

The physico-chemical parameters are important determinant of the quality of water. Temperature is one of the most important among the external factors which has a profound influence, and direct and or indirect effect on biota of an ecosystem. The average water temperature was maximum (32.47 °C ± 2.43) in July and minimum (18.15°C ± 1.02) in January. Garg et al. (2010) reported the water temperature increased during warmer

months and decreased during colder months. Islam (2007) observed the same fluctuation pattern in temperature in a Pond of Rajshahi University, Bangladesh. Turbidity is a very general term that describes the "cloudiness" or "muddiness" of water. Turbidity is caused by wide variety of suspended matter, which range in size from colloidal to coarse dispersion depending upon the degree of turbulence and also ranges from pure inorganic substances to those that are highly organic in nature. The average values of turbidity ranged between 62.25 JTU ± 6.57 minimum to 236.25 JTU ± 18.69 maximum in the month of January and August respectively. Conductivity of Paniyala pond fluctuate from 582.75 µmho/cm ± 31.76 minimum to 1164.25 µmho/cm ± 40.37 maximum in February and July respectively. ARLE (2002) reported that mineral concentrations and dilution affects the value of conductivity. It also support to present findings as high values of conductivity was registered in summer months during this period the concentrations of most of the micronutrients were at the highest level. Total solids refer to suspended and dissolved matter in water. They are very useful parameters describing the chemical constituents of the water and can be considered as a general of edaphic relations that contribute to productivity within the water body. The maximum values of total solids were observed 792 mg/l ± 28.57 in July, and minimum 327mg/l ± 24.92 in February. The concentration is high during the monsoon, which may be due to addition of solids from the runoff water. Marker (1977) has made the same observation. The average value ranges of total dissolved solids were 290.75mg/l ± 12.91 to 581.5 mg/l± 37.83 minimum in February and maximum in July month. Total suspended solids were found maximum 242.25 mg/l± 21.87 in August and minimum 31.75 mg/l± 9.76 in January.

pH is defined as the intensity of the acidic or basic character of a solution at a given temperature. The pH was found fluctuate between (8.43 ± 0.18) maximum, and (7.78 ± 0.23) minimum. The maximum value was found in the month of March and minimum value in the month of July. This is in accordance with earlier reports by Wetzel (1975) who reported that the value of pH ranges from 8 to 9 units in Indian waters. The fluctuation of pH lies in slightly alkaline range as the similar results were observed by Khanna and Bhutiani (2003). Oxygen content of water is one of the important factors, and it is very necessary for all living organisms (WHO, 2006). Dissolved oxygen concentration more than 5.00 mg/l favours good growth of flora and fauna (Das, 2000). During the present investigation the amount of dissolved oxygen ranged between minimum5.9 mg/l ± 0.10 to 7.02mg/l ± 0.31maximum in August and February respectively. The minimum values were observed during rainy months and maximum values were noticed in February in the pond. This present result was in conformity with Kumar & Singh (2000). BOD is the measure of the extent of pollution in the water body. In the present study biological oxygen demand observed maximum 4.82mg/l ± 0.23 in March and minimum 4.27 mg/l± 0.40 in October. The COD of water increases with increasing concentration of organic matter (Boyd, 1981). Chemical oxygen demand was found maximum 9.9mg/l ± 0.87 in April and minimum 8.5mg/l ± 0.45 in November. However, the increase in COD during hot period is mainly attributed to the increase in the air and water temperatures, facilitating the decomposition and oxidation of organic

matter. The similar conclusion was supported by Abdo, M.H. (2005). Free carbon dioxide refers to carbon dioxide gas dissolved in water. The average value of Free CO_2 was ranged 2.38 mg/l± 0.76 to 3.38mg/l ± 0.91 minimum in September and maximum in March. Acidity was record maximum 8.72 mg/l± 0.93 in March, and minimum 7.75mg/l ± 0.93 in September. Total Alkalinity of water is a measure of acid present in it and of the cations balanced against them. The highest average concentration was recorded 311mg/l ± 9.75 in December, and minimum 229 mg/l± 8.67 in the month of August. The low alkalinity during the monsoon may be due to dilution. Jain *et al.* (1996) also reported similar findings in their study. The total hardness value in the pond which is the sum of calcium and magnesium hardness concentrations was found to be significantly higher in the wet season max (386 mg/l ± 19.29) in July and minimum in winter (193 mg/l ± 15.45) in February. This is similar to the findings of Bhatnagar and Singh (2010). Calcium maximum 50.26mg/l ± 4.93 in July, minimum 25.32mg/l ± 2.03 in February and magnesium maximum 63.26mg/l ± 4.37 in July, minimum 31.51mg/l ± 2.01 in February. Desia (1982) reported similar trend in magnesium in Kankari lake. Chloride occurs naturally in water as man and other animals excrete chloride together with nitrogenous compounds. The water body gets chloride in it when it flows through the area when salt is deposited. The chloride ranged between (42.27mg/l± 4.93 to 31.3mg/l ± 3.57) minimum in January and maximum in August with higher concentration in summer season and lower concentration in winter season. Lendhe and Yeragi (2004) and Garg et al., (2006) have held similar view regarding seasonal variation of chloride in water.

The correlation coefficients between the physico-chemical parameters are presented in table-3. The analysis shows the high degree positive correlation between temperature and conductivity, temperature and TDS, temperature and Total Hardness, temperature and calcium, temperature and magnesium, temperature and chlorides, turbidity and TS, turbidity and TSS, turbidity and calcium, conductivity and TS, conductivity and TDS, conductivity and Total Hardness, conductivity and calcium, conductivity and magnesium, TS and TDS, TS and TSS, TS and Total Hardness, TS and calcium, TS and magnesium, TDS and Total Hardness, TDS and calcium, TDS and magnesium, TSS and Total Hardness, TSS and Acidity, COD and chlorides, Free CO_2 and Acidity, Acidity and magnesium, Total Hardness and calcium, Total Hardness and magnesium, calcium and magnesium, calcium and chlorides, magnesium and chlorides.

The analysis show the high degree negative correlation between temperature and DO, Turbidity and DO, Conductivity and pH, TS and Alkalinity, TDS and pH, TSS and Alkalinity, pH and Total Hardness, pH and magnesium, DO and calcium, BOD and chloride, COD and Alkalinity, Free CO_2 and calcium, Acidity and Alkalinity, Alkalinity and calcium, Alkalinity and chlorides. This study of fish pond indicated that positive correlation dominated significantly.

Parameter /	Temperature (°C)	Turbidity (JTU)	Conductivity (µmho/cm)	Total Solids (mg/l)	T.D.S. (mg/l)	T.S.S. (mg/l)
JAN	18.15±1.02	62.25±6.57	717±16.92	390±26.71	358.25±15.67	31.75±9.76
FEB	19.47±1.23	66.75±7.04	582.75±31.76	327±24.92	290.75±12.91	36.25±12.56
MARCH	24.47±1.58	72.25±7.16	781±37.48	428.75±30.53	390±18.58	38.75±11.92
APR	26.75±0.79	74.75±12.89	783.75±33.57	433.25±29.42	392.25±19.03	41±8.09
MAY	30.27±2.11	92.5±13.67	784.5±36.32	433.5±27.19	391.5±22.85	42±9.57
JUNE	31.55±2.08	128.5±17.45	1055.75±48.05	605±39.05	528.25±33.08	76.75±13.79
JULY	32.47±2.43	167.5±18.09	1164.25±40.37	792±28.56	581.5±37.83	210.5±19.49
AUG	28.8±1.76	236.25±18.69	924.5±32.81	706±37.51	463.25±21.89	242.25±21.87
SEPT	27.47±1.02	146.75±15.91	838±33.79	520±22.06	420.25±15.43	99.75±14.83
OCT	27.2±1.45	131.75±13.03	832±27.29	486.5±19.48	417±17.81	69.5±11.93
NOV	21.57±1.07	106.75±10.08	700.75±30.05	404.25±16.76	353.25±11.72	51±9.65
DEC	19.2±1.63	73.75±8.09	658±37.98	368.75±15.54	330±18.37	38.75±8.54

Table 1: Average value of Physical parameters of the Pond water (2008)

Parameter / Month	pH	D.O. (mg/l)	BOD (mg/l)	COD (mg/l)	Free CO_2 (mg/l)	Acidity (mg/l)	Alkalinity (mg/l)	T. Hardness (mg/l)	Calcium (mg/l)	Magnesium (mg/l)	Chlorides (mg/l)
JAN	8.22±0.38	6.75±0.32	4.6±0.28	8.92±0.28	2.55±0.57	7.93±0.89	302.5±7.98	237.5±17.86	29.33±3.97	39.89±2.97	31.3±3.57
FEB	8.28±0.17	7.02±0.31	4.77±0.43	8.95±0.37	3.23±0.87	8.48±0.81	302.5±8.93	193±15.45	25.32±2.03	31.51±2.01	33.01±4.03
MARCH	8.43±0.18	6.97±0.29	4.82±0.23	9.3±0.58	3.38±0.91	8.72±0.93	299.25±11.57	260±17.11	30.11±2.77	44.88±3.65	35.69±4.66
APR	8.32±0.45	6.55±0.37	4.77±0.38	9.9±0.87	3.24±0.46	8.67±0.98	256.25±10.79	261±18.60	32.42±3.65	43.71±4.87	37.67±3.96
MAY	8.28±0.19	6.17±0.19	4.77±0.49	9.82±0.94	3.05±0.44	8.42±0.77	270.5±6.03	260.75±13.91	35.9±4.01	41.54±4.04	40.03±4.98
JUNE	7.86±0.25	6.1±0.11	4.6±0.67	9.62±0.87	3±0.89	8.35±0.83	277.5±9.49	351±23.39	40.52±5.67	60.67±5.48	41.25±3.38
JULY	7.78±0.23	6.1±0.09	4.5±0.69	9.47±0.79	2.85±0.81	8.6±0.69	251.25±13.65	386±19.29	50.26±4.93	63.26±4.37	40.7±4.11
AUG	8.12±0.18	5.9±0.10	4.42±0.46	9.55±0.82	2.45±0.87	7.79±0.77	229±8.67	308±12.58	44.32±4.51	47.91±3.91	42.27±4.93
SEPT	8.24±0.11	5.95±0.17	4.35±0.32	9.2±0.56	2.38±0.76	7.75±0.93	281.25±12.94	278.75±19.11	40.01±3.97	43.43±2.97	38.95±3.97
OCT	8.21±0.15	6.07±0.23	4.27±0.40	8.62±0.56	2.58±0.67	7.87±0.89	295.25±11.78	277.5±9.86	37.82±3.02	44.45±4.56	35.6±2.88
NOV	8.21±0.19	6.5±0.18	4.35±0.37	8.5±0.45	2.58±0.71	7.85±0.81	303.25±10.59	234.25±16.84	32.89±4.52	36.93±3.69	35.41±3.51
DEC	8.2±0.31	6.67±0.21	4.35±0.27	8.77±0.90	2.79±0.69	8.02±0.75	311±9.75	218.5±13.26	27.14±2.99	36.6±2.98	31.68±3.86

Table 2: Average value of Chemical parameters of the Pond water (2008)

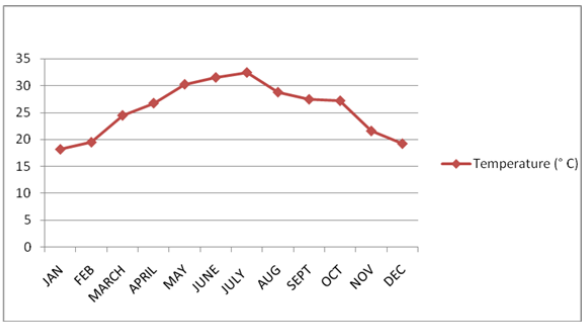

Fig.-1. Showing monthly fluctuation in temperature (°C) of fish pond

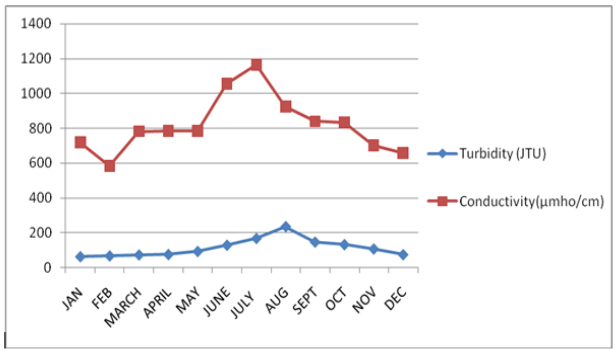

Fig.-2. Showing monthly fluctuation in turbidity (JTU) and conductivity (µmho/cm) of fish pond

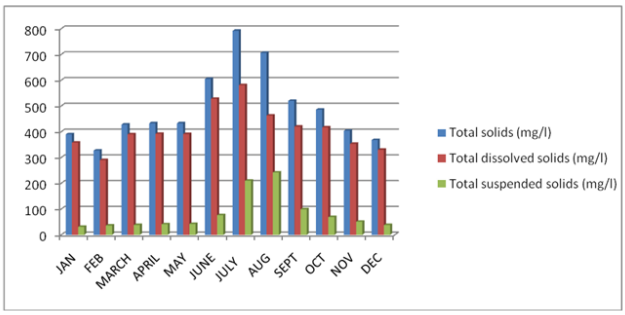

Fig.-3. Showing monthly fluctuation in Total solids, T.D.S. and T.S.S. (mg/l) of fish pond

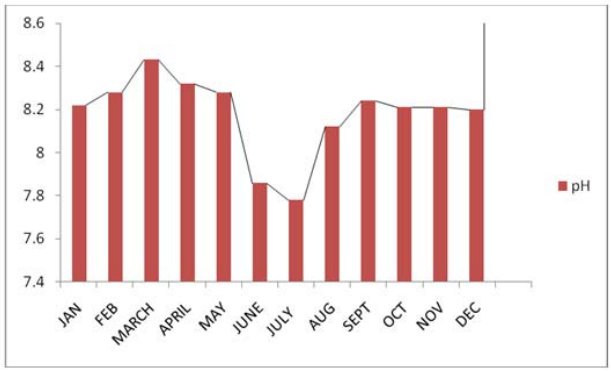

Fig.-4. Showing monthly fluctuation in pH of fish pond

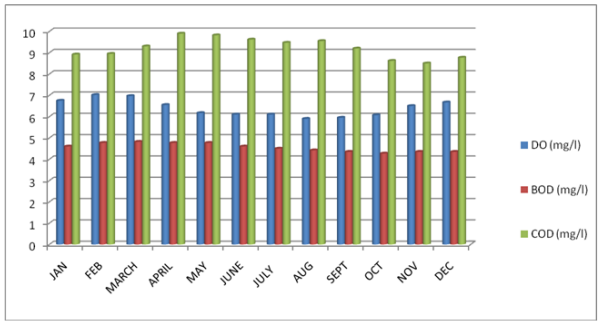

Fig.-5. Showing monthly fluctuation in DO, BOD and COD (mg/l) of fish pond

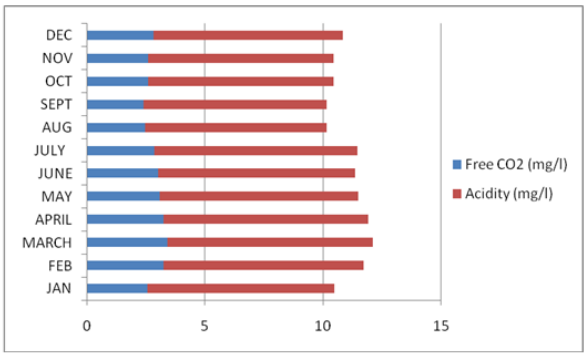

Fig.-6. Showing monthly fluctuation in Free CO_2 and Acidity (mg/l) of fish pond

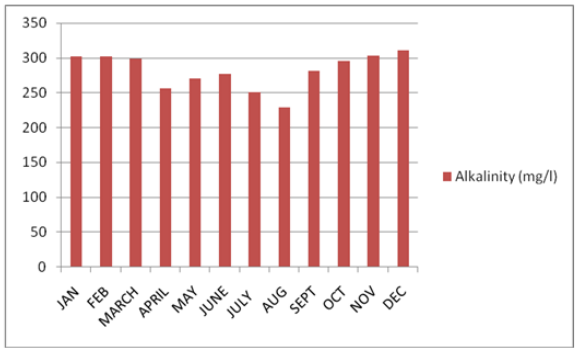

Fig.-7. Showing monthly fluctuation in Alkalinity (mg/l) of fish pond

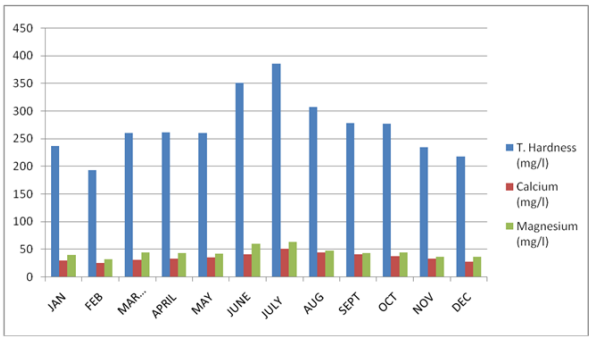

Fig.-8. Showing monthly fluctuation in Total hardness, calcium and magnesium (mg/l) of fish pond

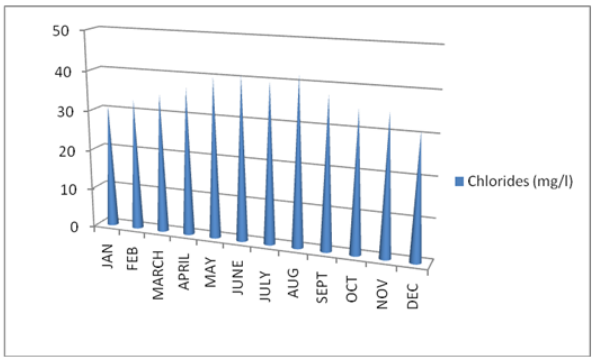

Fig.-9. Showing monthly fluctuation in chlorides (mg/l) of fish pond

	Temperature	Turbidity	Conductivity	Total Solids	T.D.S.	T.S.S.	pH	D.O.	B.O.D.	C.O.D.	Free CO2	Acidity	Alkalinity	T. Hardness	Calcium	Magnesium	Chloride
Temperature	1	0.6306051	0.8577819	0.7857238	0.8571444	0.5782843	-0.5424567	-0.7765098	0.0051562	0.675376	0.0250335	0.2361544	-0.7427521	0.8589428	0.8574184	0.8136494	0.9335032
Turbidity		1	0.67207	0.8583735	0.6766916	0.931057	-0.5374538	-0.8193956	-0.5023088	0.2127651	-0.5519629	-0.3848197	-0.7396965	0.6764017	0.8551388	0.551478	0.7502523
Conductivity			1	0.9353095	0.9999167	0.7120582	-0.8117017	-0.6927835	-0.1476719	0.4875158	-0.1268576	0.1575069	-0.6627315	0.9999038	0.9201875	0.98283	0.7862374
Total Solids				1	0.9362753	0.9143728	-0.7747295	-0.7330335	-0.2671011	0.4216899	-0.2836895	-0.0011657	-0.7788275	0.9358501	0.9583246	0.8723753	0.7980423
T.D.S.					1	0.7138865	-0.8127661	-0.6975764	-0.1575718	0.4817421	-0.1350078	0.1486258	-0.6621393	0.9999621	0.9222439	0.9818886	0.7871893
T.S.S.						1	-0.6078468	-0.6571594	-0.3510748	0.2850015	-0.4099887	-0.1735102	-0.789133	0.7130779	0.8475333	0.6073347	0.6833143
pH							1	0.5382236	0.3088984	-0.1698822	0.2376507	0.0008528	0.4026167	-0.8089764	-0.7271899	-0.8029437	-0.4958608
D.O.								1	0.5305277	-0.2986793	0.5805418	0.4106195	0.6422873	-0.6962379	-0.8585142	-0.579914	-0.7819368
B.O.D.									1	0.5821663	0.8541142	0.8213765	-0.0599535	-0.153762	-0.3423093	-0.0519024	-0.7819368
C.O.D.										1	0.4227937	0.5585129	-0.7459589	0.4841961	0.3958655	0.5023248	0.7056058
Free CO2											1	0.9313764	0.0876675	-0.1305818	-0.3739658	-0.0042986	-0.0641131
Acidity												1	-0.1275123	0.1522254	-0.0889352	0.2617044	0.1160743
Alkalinity													1	-0.6634642	-0.7538811	-0.5834029	-0.8456492
T. Hardness														1	0.9215205	0.9822915	0.7885936
Calcium															1	0.8324747	0.8430607
Magnesium																1	0.7197871
Chlorides																	1

Table-3: Correlation coefficients of different physico-chemical parameters of pond water

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Variation of fluoride and correlation with alkalinity in groundwater of shallow and deep aquifers

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Abstract

Fluoride in water is an essential element for human beings and its deficiency as well as high concentration both is injurious to human health. It is required for the protection against dental caries and weakening of bones. Groundwater in shallow aquifers that supply water to dugwells in and around Dhampur, Bijnor district of Uttar Pradesh, has higher concentrations of fluoride (F) than those of borewells from deep aquifers. Factors for variation in fluoride content between the two aquifer water types are discussed. The relative merits of the shallow water for potability are pointed out with respect to fluoride concentrations and public health. Fluoride occurs in almost all natural water supplies. Fluorides in high concentrations are not a common constituent of surface water, but they may occur in deterimental concentrations in ground waters.

Keywords: *Flouride* | *Groundwater* | *Aquifers* | *Variation* |

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Introduction

The occurrence of high fluoride concentrations in groundwater is a problem faced by many countries, notably India, Sri Lanka and China, the Rift Valley countries in East Africa, Turkey and parts of South Africa. Fluoride epidemic has been reported in as many as 19 Indian states and Union Territories. India is one among the 23 nations in the world, where fluoride contaminated groundwater is creating health problems. The state of Art Report of UNICEF confirms the fluoride problem in 177 districts of 20 states in India. The high fluoride levels in drinking water and its impacts on human health have increased the importance of defluoridation studies (Adler, 1970; Bhussry, 1970 and EPA, 1975). The magnitude of the problem is sinking in and effects are being made towards defluoridation of drinking water, combating the debilitating fluorosis and taking steps to prevent and control the disease (AMA, 1975; Chand, 1999 and Hodge, 1965).

Fluoride is well recognized as an element of public health concern. Fluoride is present universally in almost every water (higher concentrations are found in groundwater), earth crust, many minerals, rocks etc. It is also present in most of everyday needs, viz.

toothpastes, drugs, cosmetics, chewing gums, mouthwashes and so on (Subha Rao, 1997 and Thatte, 1994). Though a small amount of it is beneficial for human health for preventing dental carries, it is very harmful when present in excess of 1.0 ppm. World Health Organization (WHO) and IS: 10500 recommend that the fluoride content in drinking water should be in the range of 1.0-1.5 ppm. An intake of more than 6 ppm of fluoride results in multidimensional health manifestations, the most common being dental and skeletal fluorosis (Hubner, 1969 and Ramamohana, 1974). Higher concentration of fluoride also causes respiratory failure, fall of blood pressure and general paralysis. Loss of weight, anorexia, anemia, wasting and cochexia are among the common findings in chronic fluoride poisoning. Continuous ingestion of non-fatal dose of fluorides causes permanent inhibition of growth. Fluoride ions inhibit a variety of enzymes often by forming complexes with magnesium ions and other metal ions (Ramesam, 1985; Rao et al. 1973 and Subha Rao, 1992).

Bijnor occupies the north-west corner of the Moradabad Division and is a roughly triangular stretch of country with its apex to the north. The western boundary is formed throughout by the deep stream of the river Ganges, beyond which lie the four districts of Dehradun, Saharanpur, Muzaffarnagar and Meerut. The Dhampur is a municipal board in Bijnor district in the state of Uttar Pradesh, India. One of the largest sugar producers in India, Dhampur Sugar mills, is located here. Dhampur's main economy is based on agriculture, mainly sugarcane, wheat, and paddy. Hand-woven textile items produced by the weavers living in surrounding areas are known for their utility and designs.

Site location and Climatology

Dhampur is situated, between latitude 29°19′N and 78°31′E and longitude 29 32°N and 78 52°E at 216 meters above the sea level (Figure 1). The study area has an average monthly temperature varying 41.9°C maximum in summer and 3.2°C in winter. Average weather condition allow to recognize six well-marked traditional seasons, i.e. spring (March-April), summer (May-June), monsoon (July-August), sharada (Sep-Oct), hemanta (Nov-Dec) and winter (Jan-Feb). The average annual rainfall variation is between 1122 and 1054 mm/year.

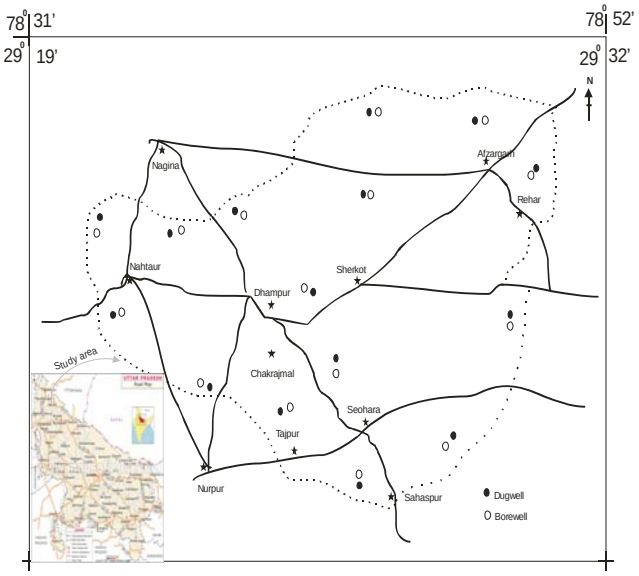

Figure 1. Base map and location map of dugwells and borewells in and around Dhampur, Bijnor

Geology

Some lethofacies of the deposits of Ganga Plain, are Mottled Silt, thick sheet like or lensoid units of well-sorted silt with little clay fraction. It represents sheet flow deposits on the higher sloping surface (Soman, 1977; Appelo and Postma, 2005), Variegated Clayey silt, thick, highly mottled clay rich sediment with intense burrowing showing clay coated sand grains, small mud pebbled, furriginization and ferruginous nodules. This represent diagenetically modified sandy silt or silty, sand sediments, which can be laterally traced for 50-100m (Lehr et al. 1980). The shallow aquifer yields groundwater at an average rate of 20 m3/day through dugwells and the deep aquifer has average yields of 105 m3/day day through borewells, due to variations in their hydraulic conductivities (Subba Rao, 1992).

Objectives and Scope

In view of the increased interest in recent years in fluoride (F) concentrations in groundwater and impact to human health, the present study is focused on factors determining F levels in the groundwater of shallow and deep aquifers in and around Dhampur, Bijnor district of Uttar Pradesh and the identification of appropriate aquifer zones for fluoride-safe drinking water.

Material and Methods

Thirty samples, 15 each from shallow dugwells and deeper borewells in close proximity, were collected for comparative study and monitoring in May 2008. The dugwells range in depth from 2 to 14m and the borewells range from 20 to 60m with averages of 9 and 34m respectively. Samples were drawn with a precleaned plastic polyethylene bottle. Prior to sampling, all the sampling containers were washed and rinsed thoroughly with the groundwater (Brown et al. 1974 and Soman, 1977). pH was measured using digital meter immediately after sampling. The fluoride concentration was determined by Ion-Selective Electrode method. This is the most convenient method for estimation of Fluoride, down to 10-5M (0.2 mg/lit), which can be stretched to 10-6M under optimum conditions. It is based on potentiometric measurements with a membrane electrode consisting of a single crystal of Europium doped Lanthanum fluoride, LaF3. The purpose of Eu doping is to improve electrical conductivity (APHA, 1998; Merck, 1974).

Result

Table:1 shows the concentration of fluoride in ppm and pH of groundwater from dugwells and borewells in the study area. For the shallow aquifers of study area, pH ranged from 7.1 to 8.2 whereas pH values for deep aquifers were ranging between 6.9 and 8.1. The amount of fluoride ion in all shallow aquifers ranged between 0.5 ppm and 1.4 ppm whereas in deep aquifers ranged between 0.3 ppm and 0.9 ppm. The observed ranged of fluoride concentration in shallow aquifers are comparatively high as compared to deep aquifers. Fluoride concentration at all shallow and deep aquifers are almost similar for all the study area. Critical analysis of data of fluoride concentration clearly indicates that the deep aquifers are deficient of fluoride at all the study area.

Elemental fluorine plays a vital role in higher life forms, especially in the skeletal systems. Both deficiency and excess of F might be harmful. Effects associated with the impact of the ion on human health greatly depend on total intake through various media such as water, air and food. For instance, the common

food stuffs have fluorine contents as follows: milk 0.07 to 0.22 ppm, wheat 0.05 ppm, rice 0.7 ppm, eggs 1.2 ppm; tea 3.2 to 178.8 ppm, garlic and onion contain 10 to 17 ppm (Kariyanna, 1987). Under these circumstances, it is advisable to consume waters having a low concentration of F to prevent fluorosis problems. The desirable limit of F in water for drinking purpose is 0.6 to 1.2 ppm, while the optimal range for it in the present study area as per temperature conditions (Public Health Service, 1962) is 0.7 to 0.8 ppm. Therefore, the ideal concentration of F may be considered to be 0.6 to 0.7 ppm. Since nearly 73% of the deep aquifer water has an F concentration between 0.6 and 0.7 ppm compared to the shallow aquifer water, the former would be more suitable than the latter for drinking purposes.

By analyzing the data it has been found that nearly all the fluoride concentration had pH level more than seven that means all the pH in the alkaline side (Table:1) for both the aquifers. Correlation analysis had been carried out to find out correlation coefficient value (Table: 1). By analyzing the data it was found that correlation value is 0.119723 in shallow aquifers and correlation value has been found in deep aquifers -0.066672. The graph has been plotted showing the basicity is the major factor for fluoride increase in the ground water show in Figure 2 and 3. Mean maximum average value has been calculated in the table for fluoride and pH value (Table: 2).

Sample Nos. shown in figure	pH	Fluoride (F-)	Correlation Value	Sample Nos. shown in figure	pH	Fluoride (F-)	Correlation Value
1 D	7.8	1.1		16 B	7.6	0.6	
2 B	7.1	0.7		17 D	7.4	1.3	
3 D	8.0	1.0		18 B	7.5	0.9	
4 B	7.5	0.6		19 D	7.2	1.1	
5 D	8.2	1.4		20 B	7.1	0.7	
6 B	8.1	0.8		21 D	8.2	1.1	
7 D	8.1	1.2		22 B	7.5	0.6	
8 B	7.4	0.7		23 D	7.9	1.0	
9 D	8.0	1.1		24 B	7.2	0.7	
10 B	7.5	0.8		25 D	7.1	1.1	
11 D	8.2	0.5		26 B	7.4	0.6	
12 B	8.0	0.5		27 D	7.4	0.8	
13 D	8.0	1.2		28 B	7.5	0.5	
14 B	6.9	0.7		29 D	7.3	0.7	
15 D	7.5	0.9		30 B	7.4	0.3	
			0.119723				-0.066672

D= Dugwell B= Borewell

Table 1. The concentration of fluoride in ppm and pH of groundwater from shallow and deep aquifers in the study area

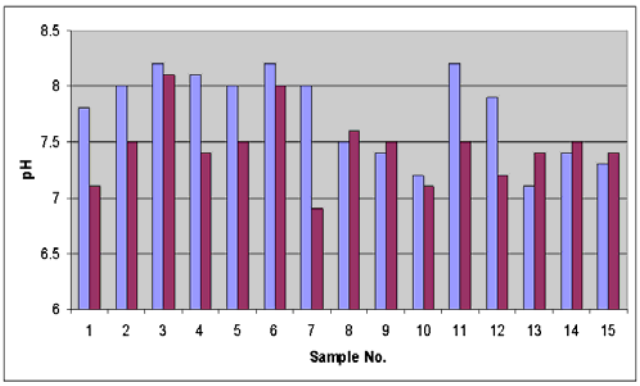

Figure 2.Graphical presentation of pH in shallow and deep aquifers

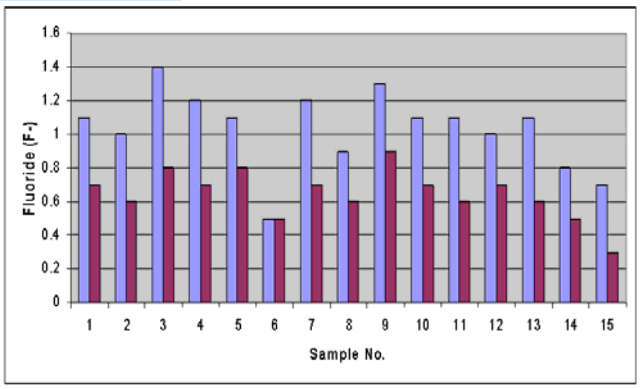

Figure 3.Graphical presentation of Fluoride concentration in shallow and deep aquifers

	For pH		For Fluoride	
	Shallow Aquifers	Deep Aquifers	Shallow Aquifers	Deep Aquifers
Sum	116.3	111.7	15.5	9.7
Average	1.0	4.4	5.3	4.25
Max	8.2	8.1	1.4	0.9
Min	7.1	6.9	0.5	0.3

Table 2. Mean Maximum and Average value for pH and Fluoride

Conclusion:

It can be concluded that fluoride-bearing water are usually high in the alkalinity and low in hardness and chloride, sulphate (Thergoankar and Kulkarni 1971). In mineralogical study of this area, we found that fluoroapatide and biotite micas contain fluoride ion. It may be because that apatite may perhaps exchange some of its hydroxyl ion for fluoride. Presence of high bicarbonates contributing to the alkalinity can also play an important part in the mineralization process (Thergaonkar et al. 1971 and Wodeyar, 1996). Similar studies in other fluoride problem areas would help to identify safe aquifer zones for drinking water. However, the borewells sampled should tap the fracture zones only. A few exploration deep borewells are also advisable where even the shallow fracture zones which are in close proximity with the weathered zones are sealed to avoid the effects of vertical leakage. It is also recommended to compare groundwater from borewells in outcrop areas (no weathered zone) to areas with weathered zones to understand the behavior of fluoride concentrations. Such studies will help solve the fluoride problem in groundwater by using hydrogeological and geochemical information for well placement rather than spending huge sums of money on alternate supply schemes.

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Understanding biodiversity – future's need

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Abstract

Biological diversity must be treated more seriously as a global resource, to be indexed, used and above all preserved. The diversity of life forms is so numerous that we have yet to identify most of them, these diversity are the greatest wonder of this planet. Increase in knowledge relating to the definition, identification and quantification of biodiversity, the information that does exist tells a compelling story about the severity of the problem of loss of biodiversity on the earth. There are many areas where human activity has its significant impacts on biodiversity whether its population or pollution. Three circumstances conspire to give this matter an unprecedented urgency. First, exploding human populations are degrading the environment at an accelerating rate, especially in tropical countries. Secondly, science is discovering new uses for biological diversity in ways that can relieve both human suffering and environmental destruction. Thirdly, much of the diversity is being irreversibly lost through extinction caused by the destruction of natural habitats, again especially in the tropics. Besides this biodiversity loss can also effects the environmental economics which in turn effecting the global economy. Overall, we are locked into a race. We must hurry to acquire the knowledge on which a wise policy of conservation and development can be based for centuries to come.

Keywords: *Biodiversity* | *Future Needs* | *Genetic* | D*iversity* |

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Introduction

The word biodiversity includes the wild and domesticated population, communities and ecosystem of living organisms present on Earth. In other words it may be defined as the variety and variability of life present on Earth. It is estimated that about 100 million species exists on earth but out of these only 1.7 million species of plant, animals and microorganisms have been discovered so far.

Generally Biological diversity involves three concepts:-

1. **Genetic Diversity**: It mainly refers to the variation of genes within the species

i.e. the total number of genetic characteristics within the specific species, subspecies or a group of species.

2. **Species Diversity**: It mainly refers to the number of species present in particular area. It has further three aspects which includes Species richness i.e. the total number of species, Species evenness i.e. the relative abundance of species and Species dominance i.e. the most abundant species.

3. **Ecosystem Diversity**: It mainly refers to differences between ecosystem types and the diversity of habitats and ecological processes occurring within each ecosystem type.

Our mother planet earth consists about 3,00,000 green plants, 8,00,000 insects, 23,000 fishes, 9000 birds ,6,500 reptiles, 4100 mammals and few thousands microbes. India, one of the 12 mega biodiversity country contains approximately 850 bacteria, 23,000 fungi, 25000 algae, 1600 lichens 2664 bryophytes, 1022 pteridophytes, 64 gymnosperms, 15,000 angiosperms, 53,430 insects, 5050 molluscs, 2,546 Pisces, 204 amphibians, 1228 Aves and 372 identified mammalian species (Pandey *et al*., 1996).

Values of Biodiversity

Biodiversity has enormous economic and aesthetic value and is largely responsible for maintaining and supporting overall environmental health. Human beings have long depended on resources for food, medicines and materials as well as for recreational and commercial purposes such as tourism and fishing. Biodiversity is an essential requirement for the maintenance of global food supplies, including plants produce, animals and fish. Besides this biodiversity also helps in maintenance of our environment

Today frequent articles in research magazine, Newspaper and discussion on media regularly attracted the public attention towards the disappearance of biological diversity and it's conserving issues.

Threats to Biodiversity

With the elevation in living standards of human beings, nature is being badly affected. Species extinction is one of the fastest growing problems related to our environment. But the difference between species extinction and other ecological problem is that species extinction is irreversible i.e. once the particular species is extinct it is forever. Species are now vanishing faster than at any other time in Earth's history. It has been estimated that between 1975 to 2015, species extinction will occur at a rate of 1 to 11 percent per decade. Aquatic species are at a higher risk of extinction than mammals and birds. Losses of this magnitude has an impact on entire ecosystem, depriving valuable resources used to provide food , medicines and industrial materials to human being.

The reasons for depletion of biological diversity ranges from overpopulation to deforestation. Increasing load on resources and our unprecedented activities are threatening the existence of other species.

Today India's ongoing economic liberalization programme may have won its many friends abroad, but it has turned out to be the worst enemy of its wild life habitats, say international environmentalists.

Across the country, essential forests habitats is being lost to mines, water logging, hydro and irrigation schemes, power plants, tea plantations etc. wheres aquatic bodies are

becoming more and more polluted under human pressure.

Deforestation, building of roads, urbanization, environmental pollution etc. are the outcome of developmental activities which ultimately destroys the habitat of particular species and as a result the species got extinct.

It is estimated that about 27,000 species are being driven towards extinction each year, which means that about 75 species are being lost every day from our planet earth. Hence we are losing our biological diversity at a faster rate and conservation strategies to protect and conserve biological diversity are necessary to maintain the balance of nature and support the availability of resources to future generation.

Conservation of Biodiversity

Conserving biological diversity has become accepted goal by many people and efforts are being done for preserving biological diversity. The growth of human population has led to decrease in biological diversity especially through habitat alterations, introduction of exotic species and through direct hunting and harvesting. If human population continues to increase, pressure will continue on endangered species and it will be a greater challenge to maintain existing biological diversity.

Why to conserve Biological Diversity?

1. All species present on earth have an inherent right to exist.
2. Sustainability is the basic principle of all types of development therefore sustainable use of biodiversity is must.
3. It is our social responsibility to preserve biological diversity so that future generation may also take benefit from it.
4. We should treat all species equal and show humanity towards them.
5. All living things are interrelated with each other through food chains, which depends upon uninterrupted functioning of natural systems, any type of interferences in these food chains may results in unbalancing of supply of energy and nutrients.

Methods of conserving biological diversity

1. Public awareness.
2. Sustainable use of Biological diversity.
3. Population control.
4. Afforestation.
5. Reduction in environmental pollution.
6. Environmental Education
7. We must discourage the purchase of products that contribute to loss of biological diversity.

Biodiversity Economics

Environmental economics can be defined as the study concerned with the impact of the economy on the environment, the significance of the environment on the economy, and the appropriate way of regulating economic activity so that a balance is achieved among environmental economic and other goals. Whereas ecological economics is the field of study deals with the relationships between ecosystems and economic system.

In case of biodiversity economics sustainability is the main aim and sustainable

development is the core environmental economics. It mainly concerns the non-declining consumption over time and focuses on development so as to meet the needs of present generation without compromising the needs of future generation. Biodiversity economics affects the environment in several ways i.e. loss of biodiversity modifies the atmosphere and hydrosphere and the diversity of wildlife inhabiting both land and water.

Further on seeing the biodiversity economics it has basically following roles:

1. To examine the costs and benefits of achieving particular objective of conserving biodiversity.
2. To examine the effectiveness of alternate policy for obtaining the objectives.
3. To examine the costs and benefits of alternative policy in conserving biodiversity.

Therefore biodiversity economics aims to put a monetary value on the environmental effects of economic decision and to provide a framework for comparing the environmental losses with economic gains.

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Influence of floral source on chemical properties of honey

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Honey is a natural sweet substance produced by honey bees from the nectar of blossoms or from secretions of living parts of plants or excretions which bees collect, transform and combine with specific substances of their own stored and leave in the comb to ripen and mature. Anonim, 1990. Honey is produced either from many flowers or from single flower. A single flower origin should assume a better quality of the product, it has a specific and well defined flavour and aroma indicating the presence of various components mainly dependent on the original sources of nectar. Besides this the honey is not 100 % unifloral because it contains various other floral sources in combination. The chemical composition of the honey shows differences depending on many factors. The most important of these is floral origin and also climatic conditions, capability of bees in making honey are the effective factors on the composition. Keskin, 1982, and the ability of the beekeepers White 1978.The diversity of the physical and chemical properties of honey like colour, flavour, moisture, protein and sugars etc. depends on the nectars and pollen of plants (Barth, 1989; White and Maker, 1980). Carbohydrates form nearly 95 % of honey. It mainly contains glucose and fructose Siddiqui and Furgula 1976. A number of investigations have been done related to physical and chemical composition of honey Anupama *et al.*, 2003; Mendes 1998; Terrab and Heredia, 2002. Melittopalynological studies are used to identify the plant source for the bees which provides the pollen and nectar source which provide the information needed in bee management and help in promoting the beekeeping development. Significant work has been reported by Suryanarayana 1992; Reddy and Reddy 2008; Tiwari *et al*. 2010.

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Material and Methods

For the present investigations seven honey samples were collected from the domesticated bee colony of *Apis cerana indica* from Nagpur region in 2009 and following parameters were studied.

Melittopalynological analysis: -10 gm of extracted honey was dissolved in 25 ml distilled water and centrifuged. The recovered sediment was treated with 5 ml of Glacial Acetic Acid and the mixture was subjected to

Acetolysis Erdtman, (1960). Three Pollen slides were prepared from each sample. The recovered pollen types were identified with the help of reference slides prepared from the local flora and relevant literature. The pollen types were identified to generic and specific levels. On the basis of presence of the pollens in the honey sample, the honey were designated as unifloral (exclusively with pollen grains of one taxa, and multifloral or mixed (with pollen grains of two or more than two taxa), Mithilesh Sharma, (1970).

Physicochemical Analysis

The water content of the sample was determined by the Digital Refractometer IHC, Bogdanov, (2002).The measurement of pH and determination of free acidity were performed at 20 ± 0.1°c on stirred solution (obtained after dissolving 10 gm of sample in the 75 ml distilled water by potentiometric titration with a 0.1M NaoH solution until pH reach 8.3 Bogdanov, (2002). Sugars was determined by using Titrematic method given by AOAC, (1990) and Indian Standards for extracted honey (1977). HMF concentration were determined according to the IHC methods.

S.No.	Sample	Moisture %	pH	Free acidity	Glucose %	Fructose %	HMF
1	*Azadiracta* honey	19.90%	4.1	22.9 meq/kg	24.50%	30.12 %	2.55mg/kg
2	*Eucalyptus* honey	19.50%	3.7	23.5meq/kg	31.50%	32.23%	-
3	*Mangifera* honey	19.80%	3.8	15meq/kg	23.80%	32.32 %	2.6 mg/kg
4	*Citrus* (Orange) honey	19.10%	3.4	22.6 meq/kg	31.70%	34.50%	5.98 mg/kg
5	*Schygium* (Jamun) honey	19.70%	3.7	30 meq/kg	29.10%	30.80%	4.50mg/kg
6	*Coriandrum* honey	19.60%	4.2	18 meq/kg	33.50%	42.52%	10.70 mg/kg
7	*Cajanus* honey	19.80%	4.1	25meq/kg	29.60%	32.50%	15.32 mg/kg

S.No.	Sample	Moisture %	pH	Free acidity	Glucose %	Fructose %	HMF
1	*Azadiracta* honey	19.70%	3.1	44.3meq/kg	21.30%	27.30%	180.6mg/kg
2	*Eucalyptus* honey	19.50%	3.4	87meq/kg	26.60%	29.20%	219mg/kg
3	*Mangifera* honey	19.70%	3.6	31.1meq/kg	21.07%	28.08%	82mg/kg
4	*Citrus* (Orange) honey	18.90%	3.2	55.5meq/kg	29.06%	30.80%	221mg/kg
5	*Schyzygium* (Jamun) honey	19.60%	3.5	55.2meq/kg	27.50%	29.80%	132.7mg/kg
6	*Coriandrum* honey	19.40%	3.9	24.2meq/kg	30.12%	40.10%	306mg/kg
7	*Cajanus* honey	19.70%	3.8	29.2meq/kg	27.30%	30.10%	420mg/kg

Pollen analysis of honey

Pollen contents of honey samples shows that the pollen types varies with the season. The

pollens of *Azadiracta indica, Eucalyptus sp., Mangifera indica, Citrus sinesis, Schyzygium cumini, Coriandrum indica and Cajanus cajan are* found to be predominant pollen and constitute the frequency more than 48 % in honey samples, so the honey samples are catagorised as unifloral honey and named after the predominant pollen type. The Cajanus honey was collected in January 09 while Coriandrum and Mangifera honey was obtained in February 09. The honey collected in March 09 showed Eucalyptus pollen predominance while that of April and May showed Citrus and Azadiracta predominance respectively.

Moisture

In the present study moisture content in the fresh honey sample ranges from 19.10-19.90 %. After storage the moisture % of honey ranges from 18.9 – 19.70 %. This result shows that there is a marginal decrease in the moisture percentage after storage. Moisture depends on the botanical origin of the sample, the degree of ripeness, processing techniques and storage conditions Instituto zooprofilalattica *et al*. (1991). All the value comes under the permitted values of IHC 2002.

PH

In the fresh honey sample pH value fell within the normal range i.e. 3.4 to 4.2 and after storage the pH ranges from 3.1 -3.9 which shows that the pH decreases after storage . The pH is of great importance during honey extraction and storage due its influence on texture, stability (Terrab *et al.*, 2003).

Free acidity

Free acidity of all fresh honey sample fell within the permitted ranges proposed by IHC (2002), with none one of them more than 50 meq/kg. The free acidity of honey samples in this study ranged from 15 to 30 meq/kg respectively (Table I) and after storage the free acidity of all samples were higher and ranges from 24.2 – 87 meq/kg (Table II).out of seven samples three samples shows the higher range which more than the 50 meq/kg. Free acidity values may indicate the fermentation of honey sugar by yeasts.

Sugars

Honey consists of mostly glucose and fructose. The actual proportion of fructose to glucose, in any particular honey, depends largely on the source of the nectar Anklam, (1998).The glucose level in seven fresh honey samples ranges from 23.80 – 33.50 % (Table-I) and after storage the value ranges from 21.07- 30.12 %. The fructose ranges from 30.12- 42.52% in fresh honey sample and after storage the values ranges from 27.30-40.10%, after storage the percentage of glucose and fructose reduces in all the samples and contained more fructose than glucose (Table-I&II); this indicates that Nagpur honeys would be less prone to granulation. Honey with high fructose to glucose ratios would remain liquid for longer periods White *et al*, (1962). The fructose/glucose ratios may have an impact on honey flavor, since fructose is much sweeter than glucose (Mead-chen, 1977).

HMF

The Hydroxymethyl furfural concentration is an important indicator of the freshness of honey .It is one of the chief products of carbohydrate degradation in food known as non-enzymatic browning. In all the seven

samples it was observed that the HMF values ranges from 1.01 to 13.5 mg/kg, this values was found to be well within the range allowed by IHC, 2002 for fresh honey sample. According to the IHC the HMF in honey sample should not be more than 40 mg/kg to 80mg/kg. After storage over a period of one year the samples shows, striking results for most of the samples where the values ranged between 82 to 420 mg/kg. The HMF formed slowly during storage and very quickly when heated.

Conclusion

The sugars i.e. glucose and fructose in the honey show a marginal decrease during the storage. The decrease in the fructose results in the reduction of texture and sweetness of stored honey. Maximum decrease in glucose was observed in *Azadiracta* honey while that of fructose decrease in Orange and Jamun honey.

The different types of honey showed difference in the increase of HMF. The increase was too high and much above the permitted level by IHC 2002, i.e. 40-80 mg/kg. *Cajanus* honey showed the greatest increased that is from 15.32-420 mg/kg. It is followed by *Coriandrum* honey that is from 10.7-306 mg/kg. The quality of honey decreases as the storage period increases. It may give negative impact in uses of honey.

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Pollen analysis of *Apis dorsata* honey collected from the Wardha, Maharashtra

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The *Apis dorsata* the largest of the four *Apis* species is distributed throughout India. On an average a single comb of *A.dorsata* yields about 25 kg of honey Mattoo, 1947; Thakkar and Tonapi, 1961; more than 50% of the total honey harvested in India is obtained from *A.dorsata* combs Phadke, 1986. The Wardha district is located in the Vidarbha region, Maharashtra and have a dense vegetation. Due to a great variety of climate and soils Wardha District is endowed with wealthy diversified natural flora which offers numerous plant species to the bees. The bees visit these plants species for the nectar, pollen or even for both of them. Evaluation of plants for their utility as source of beeforage provides the information needed to assess the potential for beekeeping in an area Mosses *et al*., 1987; Ramanujam and Khatija, 1991. Thus Melissopalynology as a branch of palynology which studies the pollen and spores in honey helps to provide the information needed for bee management and beekeeping development. It also helps to maintain the ecosystem which seems to play a crucial socioeconomic role for rural people ensuring for them an important melliferous potentials. Number of investigators have been related in pollen analysis of honey from India like Chaubal and Deodikar, 1963; Nair, 1964; Chaubal, 1982; Chaturvedi, 1983; Agashe and Rangaswamy, 1997; Bhusari *et al*. 2005

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Material and Methods

Nine samples of squeezed honeys were collected directly from the local tribal people from different localities of Wardha region immediately after its extraction. The samples were subjected to qualitative palynological analysis recommended by the International Commission for Bee Botany. (ICBB) followed by Louveaux *et al.* 1978.

Melittopalynological Analysis

1 gm of extracted honey was dissolved in 10 ml distilled water and centrifuged. The recovered sediment was treated with 5 ml of Glacial Acetic Acid and the mixture was subjected to Acetolysis Erdtman, (1960).

Three Pollen slides were prepared from each sample. The recovered pollen types were identified with the help of reference slides prepared from the local flora and relevant literature. The pollen types were identified to generic and specific levels. On the basis of presence of the pollens in the honey sample, the honey were designated as unifloral (exclusively with pollen grains of one taxa, and multifloral or mixed (with pollen grains of two or more than two taxa), Mithilesh Sharma, (1970).

Result and Discussion

Total nine honey samples were studied for the pollen analysis and twenty two plants species (Table-I) have been identified which serve as good forage for the *Apis dorsata* from the Wardha region. All samples were found to be unifloral (Fig.1) and having the predominant pollen types are *Hyptis suaveolens, Coriandrum sativum, Brassica sp., Capparis grandis, Syzygium cumin*i , *Mangifera indica*. *Delonix regia* , *Azadiracta indica* , *Syzygium cumini*. Secondary pollen in the honey samples were *Syzygium cumini, Lathyrus sativus, Sphaeranthus indicus, Cleome gynandra, Helianthus annus. Psidium guajava, Pongamia pinnata, Terminalia sp., Mangifera indica. And important minor pollen was Lagascea mollis. Tridax procumbens. Mimosa sp., Blumea sp, Sonchus oleraceus, Careya arborea* with combination of *Casearia elliptica, Albizia lebbeck* which serve as a minor pollen grain. Pollen spectra of honey revealed a variety of not only necteriferous but also necterless sources available to bees. The amount and diversity of pollen present in honey usually related to vegetation, climate and geographical location of bee hives. The study of pollen composition of the honey revealed important information on the beeforage flora of this region.

Conclusion

The present study revealed the major and minor bee forage plants Wardha district. It also indicate the availability of food source to bees in different seasons. Many non crop plants play a vital role in protecting the bee population in this region and thus helps to increase the honey production from the region. By providing this information to the farmers, local people and tribal beekeepers, help them to increase the honey production as well as crop production by pollination and also supplement the income source.

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No. of sample	Type of honey	Predominant Pollen (> 45 %)	Secondary pollen (16-45 %)	Imp. Minor pollen(< 15 %)	Minor (< 3%)
LH-01	Unifloral	*Hyptis suaveolens* 66.70 %	*Syzygium cumini* 76.48 %		
LH-02	Unifloral	*Coriandrum sativum* 56.25 %	*Lathyrus sativus* 31.50 %	*Lagascea mollis* 7.91 %	
LH-03	Unifloral	*Brassica sp*. 52 %	*Sphaeranthus indicus* 22.1 %	*Tridax procumbens* 8.2 %	
			Cleome gynandra 28.1 %		
LH-04	Unifloral	*Capparis grandis* 56.66 %	*Psidium guajava* 17.80 %	*Mimosa sp*. 17.80 %	
LH-05	Unifloral	*Syzygium cumini* 71.30 %	*Helianthus annus* 18.09 %	*Blumea sp*. 8.01 %	
LH-06	Unifloral	*Mangifera indica* 70.70 %	*Helianthus annus* 2`1.86%	*Sonchus oleraceus* 17.80 %	
LH-07	Unifloral	*Delonix regia* 54.70 %	*Pongamia pinnata* 17.08 %	*Careya arborea* 11.77 %	*Casearia elliptica* 3.09 %
					Albizia lebbeck 3.80 %
LH-08	Unifloral	*Azadiracta indica* 71.08 %	*Terminalia sp*. 29.07 %		
LH-09	Unifloral	*Syzygium cumini* 76.48 %	*Mangifera indica* 23.46 %		

Table – I: Pollen analysis of honey

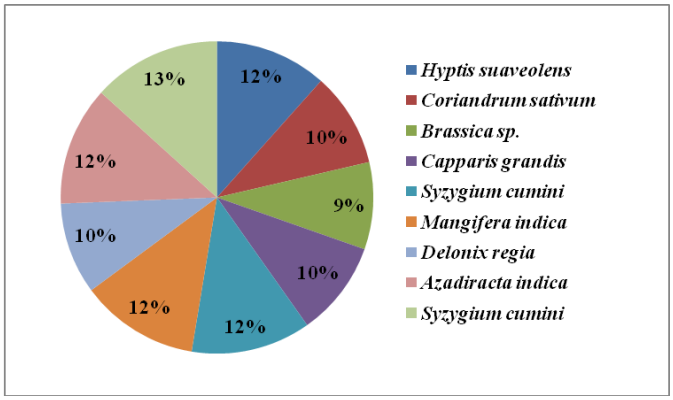

Fig. I: Composite Palynograph of Unifloral honey of Wardha

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Soil Algae at different depth in the crop field of Nagpur district, Maharashtra State

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Abstract

Soil algae play an important role in the economy of soil. Higher plants furnish the algae in its rhizosphere with more suitable condition for growth and development. Manure used for crop plants influence the algal population of the soil. The algal spores may reach up to 1 feet depth due to mixing of soil for crops. In all 83 algal taxa were found in the upper layer while its number decreases as the depth increases. There were 37 taxa in 1 inch depth, 30 in 2 inch depth, 27 in 3 inch depth, 22 in 4 inch depth, 19 in 5 inch depth and 16 in 6 in depth. There were no algal forms in vegetative condition below 6 inch deep. The culture and collection studies of soil algae of the experiment field proved the dominance of Cyanophyta in the paddy field. It may be due to non availability of height as the depth increases. The penetration of light is more in crop field due to its loose nature happened by regular ploughing. The surface layer of soil up to 1 inch contains maximum number of algal taxa and they grow luxuriantly at this region.

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Introduction

Soil algae are one of the important and major constituent of soil micro flora. It makes the composition of the soil ideal for better crop production, thus play an important role in the economy of soil. The study of rhizosphere soil algae reveals the abundance of algal population and its inter relationship between the plant root (Cherian, K.J., 2010). The microorganisms in the rhizosphere act as microbiological buffers and protect the plant from infection and pathogens. The microbial population is influenced by the plant root. These microorganisms exert a variety of effects on the growth of higher plants of which majority are beneficial. The higher plants influence the growth of specific microorganisms in the soil in their vicinity (Katznelson, *et.al.* 1948). The higher plants furnish algae in its rhizosphere with more suitable condition for the growth and development.

The type of manure used for crop plants influence the algal population in the soil. Excess of manure reduce the useful soil algae, thus makes the soil non-fertile over a period of time. The inorganic fertilizers are more

dangerous and it affects immediately. The microorganisms play a leading role in solublizing phosphorus and making it available to plants (Wani, More & Patil 1979). The plants derive benefits from mineralizing activity of some organisms.

The soil of the crop field faces mixing of upper soil layers regularly during crop and it is stagnant only during non-crop period. Thus the algal spores may reach up to 1 ft. depth in the crop field due to ploughing. Algae reach the lower layers of soil by washing down of algal forms from the surface layers. The epiterranean algae are more important than subterranean algae. The soil surface algae increase the humus by its photosynthetic activities (John 1942). Algae may function in the formation and stabilization of soil (Gray & Williams 1971). The roots of plants furnish more suitable conditions for growth and development of algae; hence they are more in number in the rhizosphere than other areas of similar depths (Gonzalves and Yalavigi 1960). Since the algae are easily washed down the passage made by roots to different depths, there may not be much difference in the rhizosphere and surface algae. Rhizosphere effect of Tea plant significantly increases the algal population in the root zone (Hadfield 1960). The roots of Pea also have stimulatory effect on algal growth (Cullimore & Woodbine 1963). The algal colonies close to the root zone were much larger than the rest of the soil. Leguminous plants favored the development of green algae while cereals favored Blue green algae.

The root samples collected from different depths carried different types of micro flora. Application of fertilizers and organic matters altered the micro flora directly or indirectly. The foliar application of certain labeled chemicals altered the root exudates like organic acid, amino acids, sugar etc. (Sankaran, 1965). Pre-treating seeds with substance like organomercurials, antibiotics etc. also alter the plant rhizosphere micro flora in the early stages of plant growth. Phorate, a systemic insecticide influences the rhizosphere micro flora of Cowpea (Visalakshi and Nair, 1979). Newman (1978) showed that the light intensity or supply of mineral nutrients to crop plant often affects the abundance of microorganisms in the soil. Algalization of the soil decrease the fungal population in rice fields (Gangawane & Saler, 1979). In Vidarbha algae of Orchard soils (Choudhary, 1977), and Blue greens of cultivated lands (Reddy 1979) were worked out by soil culture studies.

Materials & Methods

The crop fields of Nagpur district cater the cultivation of different crops like Orange, Cotton, Paddy, Wheat, Jowar, Maize, Tuar, Soya bean, Sugarcane, Vegetables etc. The type of soil varies from black cotton soil to sandy loam. The paddy field has most ideal environment for algal growth that too with vide varieties, hence for the present study paddy fields of Nagpur district is preferred. To avoid possibilities of missing any alga from observation, collection of fresh algae were done in different seasons and different locations.

Soil samples

Soil samples were collected in sterilized polythene bags from the field with sterilized scalpel & shovel from each spot of the study field. The superficial soil up to 1inch is collected to study the epiterranean algal forms. Composite soil sample up to a depth of 6 inches were collected from each plot with necessary precautions to avoid contaminations. The collected samples were brought to the laboratory and inoculated in to the culture media already prepared in 250 ml. conical flasks.

Culture media

Culture media is prepared as per the required constituents in double distilled water. Three types of media were prepared as Beneck's modified media, Chu 10 media and Allen and Arnon's media as given below.

De's (1939) modified Beneck's medium

KNO_3	-	0.2 gm
$Mgso_4\ 7H_2O$	-	0.2 gm
K_2HPO_4	-	0.2 gm
$CaCl_2\ 2H_2O$	-	0.1gm
$FeC1_3$ (1%)	-	2 drops
EOTA	-	Traces
Distilled water	-	1000 ml

Allen and Arnon's (modified) medium (Allen & Arnon, 1955, b)

$MgSO_4\ 7H_2O$	-	0.001 M
$CaC1_2$	-	0.0005 M
NaCl	-	0.004 M
K_2HPO_4	-	0.002 M

Minor elements:

$MnSO_4\ 4H_2O$	-	0.5 ppm
MoO_3	-	0.50 ppm
$ZnSO_4.\ 4H_2O$	-	0.05 ppm
$CuSO_4\ .5H_2O$	-	0.02 ppm
H_3Bo_3	-	0.50 ppm
$NH_4\ VO_3$	-	0.01 ppm
$CO\ (NO_3)_2{\cdot}6H_2O$	-	0.01 ppm
$N\ iSO_4.6\ H_2O$	-	0.01 ppm
$Cr_2\ (SO_4)_3\ K_2SO_4{\cdot}24H_2O$	-	0.01 ppm
$Na_2WO_4\ 2H_2O$	-	0.01 ppm
$TiO\ (C_2\ O_4)\ x.YH_2O$	-	0.01 ppm
Fe EDTA	-	4 ppm

Chu. No. 10 (Modified Medium

(Gerloff *et al.*, 1950)

$Ca\ (NO_3)_2$	-	0.04 gm
$K_2\ HP0_2$	-	0.01 gm
$MgSO_2\ .\ 7H_2O$	-	0.025 gm
$Na_2\ C0_3$	-	0.020 gm
$Na_2\ SiO_2$	-	0.025 gm
Ferric Citrate	-	0.003 gm
Citric acid	-	0.03 gm
A_5 solution	-	1.0 ml
Distilled water	-	1000 ml

A5 trace element stock solution

H_3B0_3	-	2.86 gm
$MnCI_2\ 4H_2O$	-	1.81 gm
$ZnSO_4\ .7H_2O$	-	0.222 gm
MoO3 (85%)	-	0.0177 gm
$CuSO_4.\ 5H_2O$	-	0.07 gm
Distilled water	-	1000 ml

The inoculated culture media were kept in racks with illumination for 12 hrs. per day.

Result and Discussion

The occurrence of a particular form at a particular sample is confirmed by soil cultures. Fresh soil sample showed the presence of algae in 10 -15 days while the dry powdered soil sample showed the presence of algae 30 - 35 days. This indicate that the appearance of algae in 10 -15 days means

they are present in vegetative form while others appeared after 30 days are grown from the spores. The spores can reach other regions due to drainage or cultivation methods. The algae which made their presence in the culture medium within 15 days after inoculation of fresh soil were considered to be present in its vegetative stage in that region.

In all 83 taxa of 30 genera could be identified through collections as well as culture studies from the experimental field. Out of the 83 taxa, 56 belongs to Cyanophyta, 18 belongs to Bacillariophyta. The genera collected with the number of species in parenthesis are Microcystis (1), Chroococcus (8), Gleothece (3), Aphanocapsa (5), Aphanothece (2), Chlorogloea (1), Oscillatoria (11), Phormidium (8), Lyngbya (3), Anabaenopsis (1), Nostoc (4),Anabaena (3), Aulosira (1), Plectonema (1),Tolypothrix (1), Calothrix (2), Hapalesiphon (1),Chlamydomonas (2), Chlorococcum (2), Scenedesmus (3), Ulothrix (5),Geminella (2), Protococcus (1), Closterium (3), Cosmarium (1), Fragillaria (1), Synedra(1), Achnanthes (1), Navicula (2), Cymbella (3), Nitzschia (2).

The Cyanophycean members were dominated over other classes of algae as it has 66.66 % of the total whereas only 22.73 % Chlorophyceae and 10.71 % Bacillariophyceae were present in the experiment field.

The result of the depth wise soil alga identified by culture studies were given in table I. In all 83 taxa could be identified from the soil upto I inch deep. Further deep soils of 1 inch, 2 inches, 3 inches, 4 inches, 5 inches and 6 inches showed 37, 30, 27, 22, 19, and 16 taxas respectively.

The presence of algae at lower layers in the soil is probably due to the washing down of algal forms from the surface layers, while John 1942 maintains that only those living algal forms at the surface can add to the supply of organic substances by photosynthetic activity, while certain Nostocaceae probably contribute to the fertility of soil by nitrogen fixation.

Since the forms identified made their presence before 15 days in the cultures medium, indicate that they were present in vegetative stage in the soil at that particular depth. More species were found in the rhizosphere at the same depth (Cherian, 2010). The loosened soil nature near the root permit the penetration of some light up to certain depth. Which helps these autotrophic organisms to grow. The loosened soil nature together with root exudates made a favourable environment in the rhizosphere. The upper layer of soil surface had 83 taxa while up to 1 inch had 37, 2 inches had 30, 3 inches had 27, 4 inches had 22, 5 inches had 19 and 6 inches had 16 forms.

The culture and collection studies of soil algae of the experiment field proved the dominance of Cyanophyta in the paddy field. It may be due to non availability of height as the depth increases. The penetration of light is more in crop field due to its loose nature happened by regular ploughing. The surface layer of soil up to 1 inch contains maximum number of algal taxa and they grow luxuriantly at this region.

Sr. No.	Name of Algal specs.	Depth in inches						
		0	1	2	3	4	5	6
	CYANOPHYTA							
1.	Microcystis stagnalis	P	-	-	-	-	-	-

2.	Chroococcus limneticus	P	P	-	-	-	-	-
3.	Chroococcus micrococcus	P	-	-	-	-	-	-
4.	Chroococcus minutes	P	P	P	P	P	-	-
5.	Chroococcus minor	P	P	P	P	P	P	-
6.	Chroococcus schizodermaticus	P	P	P	P	-	-	-
7.	Chroococcus spelaeus	P	P	P	-	-	-	-
8.	Chroococcus turgidus Var. fuscescens	P	P	-	-	-	-	-
9.	Chroococcus tenax	P	P	P	-	-	-	-
10.	Gloeothece membranacea	P	-	-	-	-	-	-
11.	Gloeothece palea	P	-	-	-	-	-	-
12.	Gloeothece samoensis	P	-	-	-	-	-	-
13.	Aphanocapsa biformis	P	-	-	-	-	-	-
14.	Aphanocapsa fonticola	P	P	P	P	P	P	P
15.	Aphanocapsa grevillei	P	P	-	-	-	-	-
16.	Aphanocapsa nivalis	P	P	P	P	P	P	P
17.	Aphanocapsa pulchra	P	-	-	-	-	-	-
18.	Aphanocapsa microscopic	P	P	P	P	P	P	P
19.	Aphanocapsa naegelii	P	P	-	-	-	-	-
20.	Chlorogloea microcystoides	P	P	P	P	P	P	P
21.	Oscillatoria animalis	P	P	-	-	-	-	-
22.	Oscillatoria curviceps Var. angusta	P	P	P	P	P	P	P
23.	Oscillatoria decolorata	P	-	-	-	-	-	-
24.	Oscillatoria grunowiana	P	-	-	-	-	-	-
25.	Oscillatoria jenensis	P	P	P	P	P	P	P
26.	Oscillatoria limosa Var. disperse-granulata	P	-	-	-	-	-	-
27.	Oscillatoria princeps	P	-	-	-	-	-	-
28.	Oscillatoria probosidea	P	-	-	-	-	-	-
29.	Oscillatoria sancta	P	-	-	-	-	-	-
30.	Oscillatoria subbrevis	P	P	P	P	P	P	P
31.	Oscillatoria terebriformis	P	-	-	-	-	-	-
32.	Phormidium africanum	P	P	P	P	P	P	P
33.	Phormidium bohneri	P	-	-	-	-	-	-
34.	Phormidium ceylanicum	P	P	P	-	-	-	-
35.	Phormidium foveolarum	P	P	P	P	-	-	-
36.	Phormidium jenkelianum	P	P	P	P	P	P	P
37.	Phormidium luridum	P	-	-	-	-	-	-
38.	Phormidium tenue	P	-	-	-	-	-	-
39.	Phormidium uncinatum	P	P	P	P	P	P	P
40.	Lyngbya aerugineo – coerulea	P	P	P	P	P	P	P
41.	Lyngbya herinymusii	P	P	P	P	P	P	P
42.	Lyngbya lachneri	P	-	-	-	-	-	-
43.	Anabaenopsis circularis	P	P	P	P	P	P	P
44.	Nostoc microscopicum	P	P	P	P	-	-	-
45.	Nostoc muscorum	P	P	P	P	-	-	-
46.	Nostoc piscinale	P	-	-	-	-	-	-
47.	Nostoc spongiaeforme	P	-	-	-	-	-	-
48.	Anabaena circinalis Var. crassa	P	-	-	-	-	-	-
49.	Anabaena laxa	P	P	P	P	P	-	-

50.	Anabaena naviculoides	P	-	-	-	-	-	-
51.	Aulosira aenigmatica	P	-	-	-	-	-	-
52.	Plectonema tomasinianum	P	-	-	-	-	-	-
53.	Tolypothrix byssoidea	P	-	-	-	-	-	-
54.	Calothrix epiphytica	P	-	-	-	-	-	-
55.	Calothrix marchica	P	-	-	-	-	-	-
56.	Hapalosiphon welwitschii	P	-	-	-	-	-	-
	Total number of Cyanophycean taxa	56	28	23	20	16	14	13
	CHLOROPHYTA							
57.	Chlamydomonas globosa	P	-	-	-	-	-	-
58.	Chlamydomonas mucicola	P	-	-	-	-	-	-
59.	Chlorococcum humicolo	P	-	-	-	-	-	-
60.	Chlorococcum vitiosum	P	P	P	P	P	P	P
61.	Scenedesmus arcuatus	P	-	-	-	-	-	-
62.	Scenedesmus bijugatus Var. bicellularis	P	-	-	-	-	-	-
63.	Scenedesmus dimorphus	P	-	-	-	-	-	-
64.	Ulothrix oscillarina	P	-	-	-	-	-	-
65.	Ulothrix subtillisima	P	-	-	-	-	-	-
66.	Ulothrix tenuissima	P	-	-	-	-	-	-
67.	Ulothrix variabilis	P	P	P	P	-	-	-
68.	Ulothrix zonata	P	-	-	-	-	-	-
69.	Geminella minor	P	P	P	P	P	P	-
70.	Geminella protogenita	P	P	-	-	-	-	-
71.	Protococcus viridis	P	-	-	-	-	-	-
72.	Closterium acerosum	P	-	-	-	-	-	-
73.	Closterium acutum	P	P	P	P	P	P	P
74.	Cosmarium granatum	P	P	P	P	P	-	-
	Total number of Chlorophycean taxa	18	6	5	5	4	3	2
	BACILLARIOPHYTA							
75.	Fragillaria brevistriata f. elongate	P	-	-	-	-	-	-
76.	Synedra affinis	P	P	P	P	P	P	P
77.	Achnanthes delicatula	P	-	-	-	-	-	-
78.	Navicula clavata	P	P	-	-	-	-	-
79.	Navicula grivilleri	P	-	-	-	-	-	-
80.	Cymbella austriaca	P	P	P	P	P	P	-
81.	Cymbella cymbiformis	P	-	-	-	-	-	-
82.	Nitzschis dissipata	P	-	-	-	-	-	-
83.	Nitzschia gracilis	P	-	-	-	-	-	-
	Total number of Bacillariphycean taxa	9	3	2	2	2	2	1
	Total algal taxa	83	37	30	27	22	19	16

Table – I: Algae Observed Depthwise In Experiment Field

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Studies on sexual dimorphism in the cyprinidae fish *Puntius ticto* (Hamilton – Buchanan) from Kumaun Himalaya, India

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Abstract

The present work is related to the sexual dimorphic nature of ornamental fish Puntius ticto (Ham.-Buch.) species from Rocky Rai stream in Kumaun Himalaya, India. This is important for taxonomy, breeding biology and pheromone biology *etc*.

Key words: *Sexual dimorphism* | *Puntius ticto* | *Rocky Rai stream* | *Kumaun Himalaya* | *India*

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Introduction

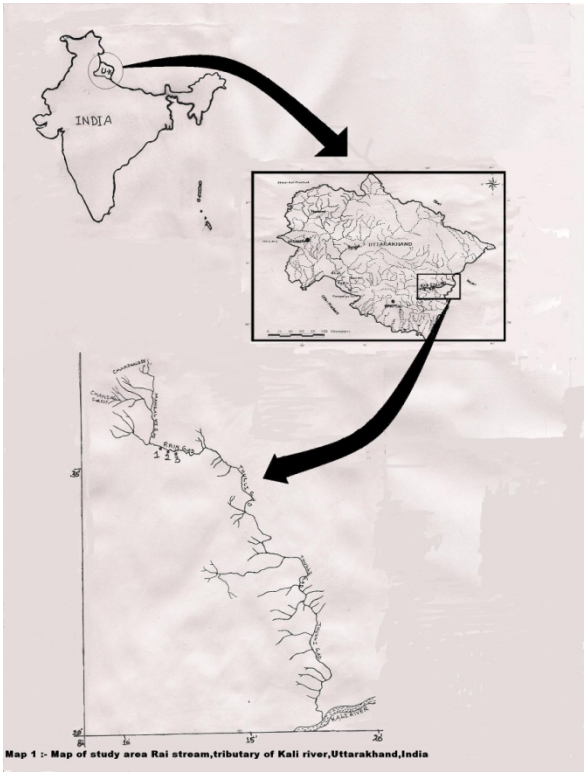
A large number of rivers, rivulets and streams from a vast network in the Kumaun Himalaya and abode a large number of indigenous fish species. The Rai stream is situated in the Central Himalayan Zone in the Uttarakhand state of India. The study of sexual dimorphism is very important in taxonomy, bionomics and breeding biology related research works. South and South East Asia is rich in small sized, often colorful species currently referred to the catchall Asian Cyprinid genus *Puntius. Puntius ticto* (Ham. – Buch.) is the most beautiful and ornamental fish among the *Puntius* species. It has been reported from various parts of Indian territory (Day. 1878; Talwar and Jhingran, 1991).

Of the large no of fish species, sexual dimorphism has been worked out only in a few species of fresh water fishes. Sexual dimorphism in fish has already been reported in different species by Thabias (1974),

Swarup and Swarup (1975), Tilak (1975), Pathani (1978), Rita Kumari and Nari (1979), Badola et. al., (1982), Inasu (1993), Tessy *et al*., (1997) and Dobriyal *et al*., (2007). Present works deals with the sexual dimorphic nature of *Puntius ticto* (Ham. – Buch.) from rocky hill stream of Kumaun Himalaya, India.

Materials and Methods

The present sampling site area study was conducted on the hill stream Rai in latitude 29º36´ N - 80º12´ E at an elevation of about 730 to 750 meters. The Rai spring fed stream is the tributary of river Kali in the upland of Kumaun Himalayan. The present work was done during the period of July 2009 to September 2009. Three sampling sites were selected in the water body of the stream. The personal collections of the fish were from different catching sites along the spring fed streams. They were preserved into 8% formalin solution and identified with the help of keys provided by Day (1878), Talwar and Jhingran (1991) and Jaya Ram (2002). Measurements were taken point to point with rounding to the nearest 0.1 mm. Total length, standard length; head length and snout length were taken from the tip of the snout to the caudal fin base, posterior opercular margin and intera orbital length respectively. Pre-dorsal, pre-pelvic and pre-anal lengths were taken from the tip of snout to the anterior base of the each fin. Lateral line is abbreviated, includes the lateral lines scales and posterior scales in the same horizontal row. Dorsal, ventral, anal and caudal fin counts and other descriptive features were noted in the present study. Local topology is used in descriptions of collecting the sampling sites (Map.1).



Map 1 :- Map of study area Rai stream,tributary of Kali river,Uttarakhand,India

Results

During the present study of the fish *Puntius ticto* (Ham.–Buch.) reach a maximum total length of 68mm for male and 70mm for female. Fish small in sized 37mm for male and 38mm for female could not be recorded in the entire study. The morphometric data on the some different body measurements is presented in the Table 1.

The meristic analysis of fish was noticed on 118 specimen and the values obtained were as follow: Fin formula D11 (3/8), P13, V9, A8 (3/5), C19, barbals are absent in mouth parts. The sales are small or medium about 20 to 25 in the lateral line; however the lateral line cases after 4 to 10 scales.

There is a dark black blotch on 15-20 scales just above the anal fin on both the sides.

During the fish biological investigations on the fish collected from Rai spring fed stream, from Kumaun Himalaya, some impression sexual dimorphism difference were observed. Our observations on the sexual dimorphism in *Puntius ticto* (Ham.–Buch.) is based on the study of 58 females and 60 male specimens, collected between, July 2009 to September 2009. The fish were segregated on the mentioned sexual dimorphic characters and dissected for conformation. We got hundred percent conformations and then decided to report it for an addition to the specific knowledge based on the study of morphometric characters.

The detailed morphometric and meristic of both male as well as female fish was studied (Table1), but no striking difference was seen. The differences are :- (1). Male with slight black blotch on the dorsal fin and some times in ventral fins, absent in female (fig. 1and 2). (2.) Upper portion of the body shinning light olive green, middle portion of the body slight blue in both the sexes; but there is dark pinkish color in the lower portion of the male on both the sides, in females there is slightly pinkish and dark yellow colour in the lower portion on both the sides. (3.) Dorsal, ventral and anal fins are dark pinkish and slight orange in male fishes but slight pinkish color shows in female fishes.


Fig. 1 :- Male Fish Puntius ticto


Fig.2 :- Female Fish Puntius ticto

Discussion

Sexual dimorphism is very significant in biodiversity assessments and also very important in biometry, breeding biology, induced breeding, breeding, pheromone biology and other related works. Well-marked structural differences are seen in the two sexes in some species, especially during the breeding season, and these are not related to copulation. In most teleost the female is larger in size than the male, and has a rounded belly during breeding season. The male have brighter color of the body and fins. Dobriyal et.al, (2007) reported that in male fish dark black shade the dorsal, ventral and anal fins but is absent in female fishes in *Puntius conchonius*. These characteristics are primary sexual dimorphic nature of *Puntius conchonius.* The upper portion of the body shinning olive green and lower portion silvery in both sexes; but there is pinkish colour in male between these two portions, which is not visible in the female fishes.

Horny tubercles are seen on the head of male in some cyprinids viz. *Tor putitora* and *Tor tor* (Pathani, 1978) and *Barilius bendelisis* (Badola et al,1982), and these are more prominent during the breeding season, this they of nature is called secondary sexual dimorphic characters. Talwar and Jhingran (1991) noticed that the arching reddish in the dorsal fin of the male *Puntius ticto* easily distinguishes the species and the dorsal fin of the female *Puntius ticto* female is pale, except for a faint rose at breeding time.

Character in ratio	Female	Male
SL in ratio of TL	1.25 - 1.46* 1.30 ± 0.04	1.25 - 1.60* 1.33 ± 0.07
CL in ratio of TL	3.14 - 4.92 4.25 ± 0.37	2.64 - 5.00 4.13 ± 0.49
PAL in ratio of TL	1.64 - 1.85 1.18 ± 0.05	1.76 - 1.95 1.81 ± 0.06
PDL in ratio of TL	1.51 - 2.73 2.40 ± 0.25	2.15 - 2.83 2.53 ± 0.15
PVL in ratio of TL	1.57 - 2.92 2.61 ± 0.28	1.95 - 2.95 2.64 ± 0.22
HL in ratio of TL	6.18 - 10.00 7.35 ± 1.02	6.16 - 9.25 7.48 ± 1.06
ED in ratio of TL	12.60 - 25.50 17.39 ± 3.86	10.25 – 19.00 14.61 ± 2.48
MBD in ratio of TL	2.66 - 4.00 3.24 ± 0.29	3.07 - 3.72 3.43 ± 0.35
Snt.L in ratio of TL	20.00 - 54.00 29.46 ± 10.95	19.00 – 39.00 26.92 ± 7.75
CL in ratio of SL	2.14 - 3.92 3.25 ± 0.37	1.64 - 4.00 3.11 ± 0.48
PAL in ratio of SL	1.20 - 1.44 1.34 ± 0.05	1.09 – 1.43 1.36 ± 0.07
PDL in ratio of SL	1.03 - 2.07 1.83 ± 0.22	1.64 - 2.08 1.90 ± 0.12
PVL in ratio of SL	1.07 - 2.29 1.99 ± 0.25	1.52 - 2.21 1.99 ± 0.15
HL in ratio of SL	4.90 - 7.60 5.61 ± 0.80	4.14 - 7.25 5.62 ± 0.73
ED in ratio of SL	9.66 - 19.50 13.30 ± 3.15	8.00 - 15.00 10.99 ± 1.83
IOL in ratio of SL	5.00 - 18.00 9.47 ± 4.81	3.83 - 7.33 6.16 ± 0.86
MBD in ratio of SL	2.40 - 2.65	1.66 - 2.81

	2.47 ± 0.23	2.53 ± 0.35
Snt L in ratio of SL	15.00 - 41.00 22.40 ± 8.15	14.00 – 29.00 20.12 ± 5.44
ED in ratio of HL	1.60 - 3.33 2.31 ± 0.51	1.33 - 2.66 1.97 ± 0.33
Snt L in ratio of HL	2.50 - 8.00 4.01 ± 1.43	2.50 - 7.00 3.61 ± 1.05
IOL in ratio of HL	1.00 - 3.00 1.62 ± 0.83	0.60 - 1.75 1.09 ± 0.25
MBD in ratio of HL	0.40 - 0.63 0.44 ± 0.07	0.33 - 0.60 0.47 ± 1.24

Table-1: Some important taxonomic characters in male and female of *Puntius ticto* (Ham.-Buch)

Inasu (1993) observed that males are larger then females of the same age group in *Tetradone travencooricus* (Hora and Nair) but Tessy and Inasu (1997) observed that in the edibal perch *Priacanthus hamrus* (Cur. and Val.) females are more than two times larger and heavier than the males of the same age group. Kurian and Inasu (1997) noticed that female are dominance is observed in *Ompak bimaculatus* (Bloch) also, since female is more or less two times larger and five times heavier than the males of the same age group but in *Horabagrus brachysoma*(Gunther) the males are found slightly larger and heavier than the females of the same age group. Arunanchalam and Johnson (2002) observed that sexual dimorphism in *Puntius kannikattiensis*, males deep black, tubercles on front of snout, and extended laterally below the eyes, also on the lower jaw. Black blotches on the body not clear. Fins and lips deep black. In female; snout was plain, and no tubercles found on snout or lower jaw. Lips are white, fins pale yellow to dull white; entire body blackish –brown, blotches distinct.

In the present study a significant sexual dimorphism was notices in the fish *Puntius ticto*. The male fish have slight black blotch on the dorsal and ventral fins, which were not found in any of the female fish. There was a well marked dark pinkish colour in lower portion of the male on both the side. In female, there is slightly pinkish and dark yellow color found in both the sides. The dorsal, ventral and anal fins are dark pinkish and slight orange in the male while slight pinkish and dark orange color found in female fishes. In the present investigation, statistical analysis of morphometric data revealed that certain characters slightly differ in male and females.

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Effect of Heavy Metal Nickel on Enzymological Parameters of *Cirrhina Mrigala*

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Abstract

The aim of the present investigation is to study the chronic toxicity of heavy metal Nickel on some enzymes of carbohydrate metabolism in tissues of fresh water fish *Cirrhina mrigala*. Fish were exposed to sublethal dose of Nickel chloride ($NiCl_2$) for 40 days. The first batch of fish was sacrificed after 20 days of exposure to sub lethal dose of nickel and the second after 40 days. The tissue extracts from liver, muscle, gills were taken and tested for the activity of key enzymes of glycolysis and Kreb's cycle. Increase in the activity of G-6-Pase in liver and gills shows increased rate of glycogenolysis to meet energy demands of body. Increase in activity of LDH and inhibition in activity of PDH and SDH in liver, muscle and gills indicate shift in metabolism from aerobic to anaerobic.

Key words: Tissues | Enzymes | Nickel | *Cirrhina mrigala*

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Introduction

Heavy metals are common persistent pollutants of aquatic ecosystem entering them through numerous diverse anthropogenic and natural sources (Moore, 1991). Many industrial and agricultural processes have contributed to the contamination of fresh water systems thereby causing adverse effects on aquatic biota and human health (Rose *et al*., 1999). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, *et al.,* 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005). Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects (Palaniappan and Karthikeyan, 2009).

Anthropogenic activities (i.e. mining, electroplating & steel plant operations) can result in nickel discharge into water and air (Galvin, 1996). The nickel is an essential element at low concentrations for many organisms; it is toxic at higher concentrations

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(Clark and Keasling, 2002). Fish residing in polluted freshwater systems are exposed to *Ni*, primarily, through the ingestion of contaminated food and sediments (Dallinger and Kautzky, 1985). The heavy metal in the tissue of fishes may cause various physiological defects and mortality (Torres *et al*., 1987). Hence, the present investigation has been carried out to study the effect of nickel on enzyme activities in the various tissues of freshwater fish *Cirrhinus mrigala* on chronic exposure for 40 days.

Materials and Method

The fish, *Cirrihina mrigala* were purchased from the local fish market having an average length 12±3 cm and weight 200±2 gms The fish were then kept in different aquaria for conduction of various experiments. The fish were acclimatized to laboratory conditions in aquaria for a few days. In one aquarium the fish were kept as control specimens given the same food and environment as that of the experimental fish except that they were not given the dose of heavy metal compound. Inorganic salt of heavy metal nickel namely nickel chloride anhydrous ($NiCl_2$) was the experimental toxicant. To observe the chronic effects of nickel, sublethal dose (1/10 concentration of 96 hr LC_{50}) of the heavy metal compound was given for 40 days. The first batch of fish was sacrificed after 20 days of exposure to sub lethal dose of nickel and the second after 40 days. During exposure period the fish were conditioned to feeding on packed fish food at the rate of 2% of body weight. The fish were fed once daily at 11am.

For the estimation of activities of enzymes of glycolysis and kreb's cycle, 10% of W/V homogenates were prepared in 0.25 M sucrose solution for tissues namely liver, gills and muscles. The homogenates were centrifuged at 1000x g for 20 min. and clear supernatant fluids were used as the source of enzymes. The activities of Lactate Dehydrogenase(LDH), Pyruvate Dehydrogenase(PDH), Succinate Dehydrogenase(SDH) enzymes were estimated by the triphenyltetrazolium chloride method of Srikantan and Krishnamoorthi (1995). The activity of Glucose-6-Phosphatase was determined by adopting the method of Swanson (1955**).** The significance of the difference between control and experimental means was calculated by Students't' test (Wardlaw, 1985).

Results and Discussion

Liver (Table-1)

A progressive increase was observed in the activity of Glucose-6-Phosphatase ranging from 38.03% to 62.65% during the entire exposure period. The activity of lactate dehydrogenase (LDH) increased after 40 days by 25.46% but decreased after 20 days by 26.16%. Reduction was observed in the activity of pyruvate dehydrogenase (PDH) after 40 days however insignificant change was observed after 20 days of exposure. The activity of succinate dehydrogenase (SDH) increased after 20 days of exposure whereas decrease of 14.68% was observed in the enzyme activity after 40 days of exposure.

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Gill (Table-2)

Progressive increase was noticed in the activity of Glucose-6-Phosphatase after 20 and 40 days of exposure by 10.17% and 37.73% respectively. The activity of lactate dehydrogenase (LDH) increased by 35.93% after 20 days and 54.00% after 40 days of exposure. Reduction was observed in the activity of Pyruvate dehydrogenase (PDH) and Succinate dehydrogenase (SDH) upto 40 days of exposure period.

Muscle (Table-3)

The activity of lactate dehydrogenase(LDH) increased initially by 17.68% after 20 days and was insignificant after 40 days of exposure. The activity of pyruvate dehydrogenase (PDH) decreased upto 40 days of exposure. The succinate dehydrogenase (SDH) activity decreased after 20 days (35.05%), was insignificant after 40 days.

On exposure to nickel, different metabolic alterations produced in different tissues varied with period of exposure. Increase in the activity of Glucose-6-Phosphatase in liver and gills in all stages of exposure shows active breakdown of glycogen reserve or increased rate of glycogenolysis to meet energy demands of body. Dange (1986a) reported increase in glycogenolysis in *Sarotherodon mossambicus* exposed for 96 hr to a variety of chemicals such as mercury, copper, naphthalene and phenol.

Increase in the activity of SDH and decrease in activity of LDH in liver after 20days shows that in fish, the rate of oxidative metabolism is increased to withstand the toxic stress. However on chronic exposure for 40 days there was significant increase in activity of LDH and inhibition in activity of PDH and SDH in liver indicating shift in metabolism from aerobic to anaerobic. Inhibition in the activity of LDH noted by Rajeshwari et al (1990) in liver of *Channa punctatus* acutely and chronically exposed to cadmium lends support to the present findings.

Anaerobic metabolism was favoured in gills (20, 40 days) and muscle (20 days) and in liver after 40 days to meet energy demands. Evidence in support to this comes from increase noted in the activity of LDH and decrease in the activity of PDH and SDH. Sastry and Rao (1981) observed decrease in LDH activity in liver, gills and Kidney of *Channa punctatus* exposed to mercury.

ENZYMES	CONTROL	20 DAYS	40 DAYS
GLUCOSE-6-PHOSPHATASE [a]	233.39±0.02	322.16±0.01***	379.63±0.01***
LACTATE DEHYDROGENASE [b]	4.79±0.04	3.16±0.03***	5.37±0.05***
PYRUVATE DEHYDROGENASE [b]	3.14±0.05	3.18±0.06NS	2.45±0.02***
SUCCINATE DEHYDROGENASE [b]	5.09±0.01	5.89±0.02***	4.30±0.07***

Values are mean ±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a]µg inorganic phosphate/mg Protein/hr
[b]µg formazon/mg Protein/hr

Table 1: Alterations in Liver Enzyme Activities in *Cirrhina Mrigala* Exposed to Nickel (Ni) For 40 Days

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ENZYMES	CONTROL	20 DAYS	40 DAYS
GLUCOSE-6-PHOSPHATASE [a]	27.72±0.05	30.54±0.04***	38.18±0.03***
LACTATE DEHYDROGENASE [b]	4.87±0.03	6.62±0.06***	7.50±0.07***
PYRUVATE DEHYDROGENASE [b]	3.73±0.01	2.06±0.03***	1.79±0.04***
SUCCINATE DEHYDROGENASE [b]	6.49±0.04	5.05±0.02***	4.79±0.04***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a]µg inorganic phosphate/mg Protein/hr
[b]µg formazon/mg Protein/hr

Table 2: Alterations in Gill Enzyme Activities in *Cirrhina Mrigala* Exposed io Nickel (Ni) For 40 Days

ENZYMES	CONTROL	20 DAYS	40 DAYS
LACTATE DEHYDROGENASE [b]	8.03±0.02	9.25±0.04***	7.76±0.19NS
PYRUVATE DEHYDROGENASE [b]	11.05±0.04	4.01±0.06***	5.30±0.06***
SUCCINATE DEHYDROGENASE [b]	4.51±0.03	3.15±0.02***	4.40±0.11NS

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[b]µg formazon/mg Protein/hr

Table 3: Alterations in Muscle Enzyme Activities in *Cirrhina Mrigala* Exposed to Nickel (Ni) For 40 Days

Conclusion

From the results obtained in the present study it can be concluded that chronic exposure of heavy metal nickel in fresh water fish *Cirrhina mrigala* led to shift in the metabolism from aerobiasis to anaerobiasis to combat the toxic stress.

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Nickel Toxicity: Biochemical Alterations in *Cirrhina Mrigala*

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Abstract

The present research is designed to determine the effect of nickel toxicity on biochemical parameters of fresh water fish *Cirrhina mrigala* on chronic exposure for 40 days. The fish were kept in different aquaria for different exposures and one aquarium was kept unstressed as control. Fish were exposed to sublethal dose of nickel chloride ($NiCl_2$) for 40 days. The first batch of fishes was sacrificed after 20 days of exposure to sub lethal dose of nickel and the second after 40 days. The levels of glucose, lactic acid and pyruvic acid were estimated in blood. Glycogen was estimated in liver. The levels of glucose and lactic acid increased in the blood while pyruvic acid in the blood and glycogen content in liver decreased. These results indicate that biochemical parameters of fish are altered on exposure to heavy metal nickel.

Key words: Biological Parameters | Nickel | *Cirrhina mrigala*

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Introduction

The global heavy metal pollution of water is a major environmental problem. (S. Senthil Murugan, 2008). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi, *et al.,* 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005). Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.,* 2002; Yousuf and El-Shahawi, 1999). The toxic metals are held to be the most dangerous, since continuous exposure of aquatic organisms to their low concentration may result in bioaccumulation and transfer to man through food web (Gaspic *et al*., 2007).

Nickel toxicity is generally low (Khangarot & Ray, 1990) but elevated concentrations can cause lethal effects in the aquatic ecosystems. Metal mining, smelting, refining, and processing along with fuel combustion and waste incineration activities release significant amounts of nickel (Ni) into freshwater habitats

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through atmospheric deposition and in liquid effluents and leachates (Chau and Kulikovsky-Cordeiro, 1995). It is also used extensively in electroplating as nickel sulphate and nickel hydroxide is used in nickel–cadmium batteries (Nanda and Behera, 1996). Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish (Tulasi *et al*, 1992). So the present research is designed to study the effect of nickel toxicity on biochemical parameters of fresh water fish *Cirrhina mrigala.*

Materials and Method

The fish, *Cirrhina mrigala* were purchased from the local fish market having an average length 12±3 cm and wt 200±2 gms The fish were then kept in different aquaria for conduction of various experiments. The fish were acclimatized to laboratory conditions in aquaria for a few days. In one aquarium the fish were kept as control specimens given the same food and environment as that of the experimental fish except that they were not given the dose of heavy metal compound. Inorganic salt of heavy metal nickel namely nickel chloride anhydrous ($NiCl_2$) was the experimental toxicant. To observe the chronic effects of lead, sublethal dose (1/10 concentration of 96 hr LC_{50}) of the heavy metal compound was given for 40 days. During exposure period the fish were conditioned to feeding on packed fish food at the rate of 2% of body weight. The fish were fed once daily at 11 am.

The first batch of fishes was sacrificed after 20 days of exposure to sub lethal dose of nickel and the second after 40 days. Glycogen in liver was estimated by the method of Hassid and Abrahim (1957). Blood from the caudal vessel of both control and experimental fish was drawn with the help of heparinized needles. The levels of glucose, lactic acid and pyruvic acid were estimated in blood. Glucose was determined by the method of Folin and Wu (1929). Pyruvic acid was determined by the method of Friedmann and Haugen (1944). Lactic acid in blood was estimated according to the method of Barker (1963). The significance of the difference between control and experimental means was calculated by Students 't' test (Wardlaw, 1985).

Results and Discussion

On chronic exposure to nickel, alterations were observed in the biochemical components of fish in the present study. The blood of *Cirrhina mrigala* showed significant increase in glucose during heavy metal intoxication after 40 days of exposure. This might be due to the vulnerable stress induced by the heavy metals that resulted in hyperglycemia. The elevated level of glucose after 40 days of exposure suggests that the hepatic glycogen is probably the source of hyperglycemia in fish. Depletion of liver glycogen after 40 days of exposure corresponds to dramatic increase in blood glucose level suggesting that some of the hepatic glycogen via intermediate glucose-1-phosphate is converted to glucose and that this glucose enters the general circulation (Hinstal *et al*, 1983). R. Vinodhini *et al* (2008) also reported increase in blood glucose level after exposure of combined metal solution of Cd, Pb, Cr and Ni for 32 days in *Cyprinus carpio*.

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Almeida *et al* (2001) also recorded that heavy metal exposure increases glucose content of blood because of intensive glycogenolysis and synthesis of glucose from extra hepatic tissue proteins and amino acids.

Glycogen level decreased in liver after exposure of 40 days. However change in glycogen content was insignificant after exposure of 20 days. Lowering of hepatic glycogen reflects reduced glycogenesis and increased glycogenolysis. Evidence in support of this comes from the increased blood glucose level. Depletion of glycogen in liver may further be correlated with a high demand of glycogen for excess energy requirements. S.S.Vutukuru (2005) also found decrease in glycogen content of liver in *Labeo rohita* exposed to chromium suggested that it may be due to enhanced utilization of glycogen as an intermediate source to meet energy demands under metallic stress. Decreased glycogen content may also be due to prevalence of hypoxic or anoxic conditions, which normally enhances glycogen utilization (Dezwaan. A.et al (1973). According to Chandravathy Mary et al (1995) under hypoxic conditions, the animal derives its energy from anaerobic breakdown of glucose, which is available to cell by the increased glycogenolysis.

It was observed that after 40 days the fish suffered a severe respiratory stress as evident in the depletion of glycogen and pyruvic acids with elevation of lactic acid levels. On nickel exposure, decreased pyruvate level suggests inhibition in glycolysis and enhanced pyruvate utilization by fish under chronic stress. Vineeta Shukla and Shastri (1998) also recorded decrease in pyruvic acid content of blood in *Channa punctatus* exposed to cadmium (Cd). Similar elevation in lactic acid level has been recorded in *Channa punctatus* on exposure to Chromium by Sastry and Tyagi (1982). The increased lactic acid content relative to pyruvic content may indicate that the oxygen supply to the tissue is not adequate for the normal metabolic functions.

PARAMETERS	CONTROL	20 DAYS	40 DAYS
BLOOD Glucose [a]	0.13±0.01	0.17±0.01**	0.41±0.05***
Lactic Acid [a]	0.09±0.01	0.07±0.01**	0.17±0.03***
Pyruvic Acid [a]	0.77±0.03	0.46±0.02***	0.58±0.01***
LIVER Glycogen [b]	0.70±0.05	0.74±0.01NS	0.25±0.03***

Values are mean±SD; n=6, NS= not significant
*significant, *p<0.05, **p<0.01, ***p<0.001
[a] mg/ml of blood
[b] mg/gm wet weight of tissue

TABLE 1: Alterations in Biochemical Parameters In *Cirrhina Mrigala* Exposed To Nickel (Ni) For 40 Days

Conclusion

It can be concluded from the results of present study that fish suffered severe respiratory stress due to nickel toxicity that led to alterations in biochemical parameters.

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Counteractive impacts of plant growth regulators over uv-b radiation damage on certain physiological and biological aspects in rice crop

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Abstract

The aim of study was to evaluate the appropriate concentrations of plant growth hormones from various concentrations over the UV-B damage on *Oryza sativa* in case of chlorophyll isolation. Seeds of *Oryza sativa* were grown in laboratory for the seed germination and seedling growth and then sown in field plots (A, B, C, D) with appropriate concentrations of plant hormones for the isolation of chlorophyll-a, chlorophyll-b,protochlorophyll. Plot-A of rice crop was treated as control and neither sprayed with growth hormones nor exposed to UVB radiation. Pot-B was treated with UV-B radiation (3-hrs. daily) only. Plot-C was sprayed with IAA concentration of (10^{-7} M), plot-D was sprayed with Kn concentration of (10^{-5} M), along with UV-B radiation in *Oryza sativa*. IAA was found most effective in (10^{-7} M), Kn in (10^{-5}M) in crop of *Oryza sativa* and observed enhancement in the chlorophyll at the germinating seedling stage in the laboratory and field study till to maturity of crop.

Key words: *Oryza sativa* | Plant growth regulators | IAA Kn | Chlorophyll a | Chlorophyll b | Protochlorophyll | UV-B radiation

Introduction

The ozone layer is found at altitudes between 10 and 30 kilometers with a maximum concentration from 19 to 23 km. The total height of the ozone column above any spot on earth is quite small. At standard temperature and pressure, the entire stratospheric ozone layer would have a depth of only 0.3 cm. the depletion of stratospheric ozone caused by increasing human activities have led to an elevation of Ultraviolet-B radiation at high altitudes. Since ambient levels of Ultraviolet-B (UV-B) radiation in the tropics are already high, any further enhancement in UV-B could be of considerable importance in these regions. It may significantly alter plant ecosystems by reducing the productivity of several

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economically important crop plants. India lies in the low ozone belt and is expected to receive high flux of UV-B radiation, which may affect plant growth and metabolism. The increase of ozone depletion raises concerns since the deleterious effects of UV-B radiation have been observed in various plant species (M. Tevini and A.H. Teramura, 1989). Some plants react to UV-B radiation by developing protective mechanisms such as synthesis of UV-B absorbing pigments and thickening of the leaf (Tevini *et al*., 1991). Several recent experiments have shown that UV-B radiation causes increase in the level of cellular reactive oxygen species generating oxidative stress and it is generally accepted'that the mechanism of UV-B toxicity involves oxidative damage (Mazza *et al*., 1999).

Tevini *et al*., (1990 a and b; 1991) observed reduced germination, seedling growth and leaf size in sunflower and maize under high UV-B concentration stimulus. Germination and growth reductions were also found in wild type and stable phytochrome-deficient mutant of cucumber (Ballare *et al*., 1991). The molecular reasons for germination and growth reductions can be attributed to changes in DNA or phytochromes can affect germination and growth by altering their concentration in the growth sensitive tissues and by changing phytochrome dependent processes. Growth in length of root and shoot is related to IAA (indole acetic acid), which absorbs the UV-B range and is readily photo-destroyed by UV-B in vitro and in vivo as shown in sunflower seedlings under low white light conditions. Furthermore, the plastic epidermal cell wall extensibility, which is enhanced in auxin induced elongation, growth was also reduced (Ros, 1990). Peroxidase activity, which can reduce elongation at high activity by different mechanism, was enhanced in UV-B irradiated sunflower (Ros, 1990) and sugarbeet plants (Panagopoulos *et al*., 1990). Another phytohormone, ethylene, which changes elongation of radial growth, is produced to a greater extent in UV-B irradiated sunflower seedlings (Ros, 1990). In UV-B exposed cucumber and bean seedlings, growth could be stimulated by gibberellins (Saile-Mark, 1993; Dhingra *et al*., 2003).

Materials and Methods

The present study was undertaken at the field of R.C. U. Government Post-Graduate. College, Uttarkashi during. The proper study site was located at Purikhet campus of the college near river Bhagirathi. Four plots measuring 1 x 1 in each were fenced by barbed wire to avoid any biotic interference. Certified seeds of cereals crop *Oryza sativa* were procured from extension branch of Indian Agricultural Research Institute, New Delhi.

General Experimental Design: - During laboratory studies following sets were taken into consideration:

Control: Seeds were soaked for 24-hr. in distilled water and placed on moistened filter paper in Peridishes.

UV-B: UV-B radiation was supplied for 3-hr daily by sunlamps (300 W), filtered with quartz interference filters (320 run, ORIEL, USA).

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Growth Regulators: Test solutions of IAA and Kn were prepared in three concentrations viz. 10^{-7}, 10^{-6}, 10^{-5} M (Molarity). Seeds of *Oryza sativa* were soaked for 24-hr in different concentrations of sd k growth regulators. Soaked seeds were placed in paired Petridishes lined with moistened filter paper. One set of Petridish containing soaked seeds was allowed to grow without any UV-B exposure.

Growth Regulators + UV-B: In second set-one from each concentration of different growth regulators was treated with UV-B radiation, for 3-hr daily.

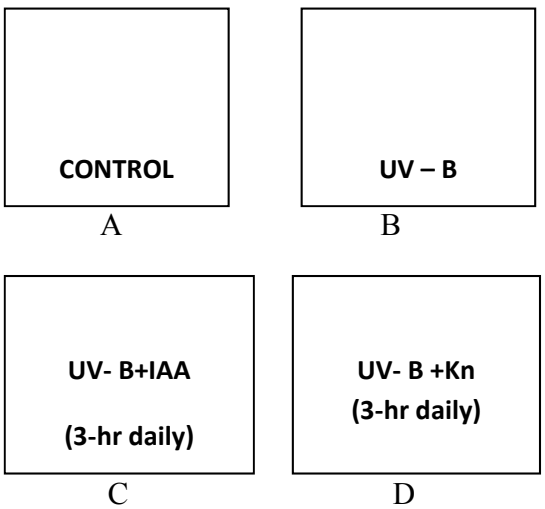
During field study, both the crops were grown in field and the plot was divided by black paper. sheets into four blocks. Each field block was given treatment as:

1. Plot A was taken as control. No treatment was given to the crop of this plot.
2. Plot B was irradiated with 3-hr daily UV-B radiation (24.23 Jm211) by Sunlamps (300 W) filtered with quartz interference filters (320 tim, ORIEL, USA).
3. Plot C was sprayed with IAA ($10^{-.6}$ M concentration) daily alongwith 3-hr supplemental UV-B radiation using the same source.
4. Plot D was sprayed with Kn (10^{-6} M concentration) daily alongwith 3-hr supplemental UV-13 radiation.

General experimental design may be summarized as:

Treatments Concentration	Control	UV-B	IAA			Kn			UV-B+IAA			UV-B+Kn		
		(3-hr)	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}	10^{-7}	10^{-6}	10^{-5}

Treatment of plots in field conditions:



The field for cultivation was prepared before sowing of seeds as proposed by Dhasmana (1984). Pre-soaked seeds of the crops were sown in the experimental plots. The general experimental plan for different treatments was laid after full germination of both the crops (Kumar, 1981; Dhasmana, 1984; Ambrish, 1992; Dhingra, 1999; Neeta Bhatt, 2002).

Chlorophylls:

Fresh leaves (500 mg) were homogenised with 80% acetone, centrifuged at 4000 rpm for 5 minutes. Filtrate was taken out and final volume was made 100 ml, using 80% acetone.

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Optical density was read at different wavelengths viz. 626, 645, 663 mm with the help of Systronics Digital Spectrophotometer. The Chlorophyll COl1tefltS were estimated by the formula given by Koski and Smith (1948) which are expressed below:

Chl a,	mg/l =	12.67 A	663 - 2.65 A	645-0.29	626
Chl b,	mg/l =	23.60 A	645 - 4.23 A	663-0.33	626
Protochl,	mg/l =	29.60 A	626 - 3.39 A	663-6.75	645

Anthocyanins

Anthocyanins during germination of seeds or seedlings were extracted by using the modified method of Mancinelli *et al.* (1975). 500 mg, fresh weight of seedlings was grinded in methanolic HCl (80 ml methanol, 20 ml water, 1 ml HCI). Homogenised tissue was transferred into glass stoppered bottle using appropriate amount of methanolic HCl, stored them overnight in refrigerator. It was centrifuged at 4000 rpm and collected in conical flask. Final volume was made 25 ml with methanolic FTC. Absorbance was taken at 530 nm and 660 nm, with the help of Systronics - Digital UV-spectrophotometer. Calculation was determined by using the formulae given by Mancinelli *et al.* (1975).

As = at 530 — 1/3 A at 660

Where As = Anthocynins

A = Absorbance

Enzymes:

1. **Protease:**

Extraction

Ptotease enzyme was extracted in laboratory conditions from germinating seeds of different treatments of both the crops. One gram of germinated seeds were homogenised in chilled Tris-HCI bufferntrifuged at 5000 rpm for 5 minutes and then supernatant was used as enzyme source. The volume of supernatant was made 25 ml by adding Tris-HCI buffer. All the operations were carried out at 4 to 5°C (Sadasivam and Manickam, 1996).

Assay

Protease activity in extracted material was measured by modified method of Green and Neurath (1954). One ml of extracted material in TrisHC1 buffer, extracted earlier and preserved at 4°C, one ml Of protein solution and one ml of Tris-HC1 was incubated at 40°C for 1 hr. One ml of TCA was added to the above and kept in freezer for 3-hr. After that, the whole solution was centrifuged to get clear supernatant. In supernatant solution, 1 ml of 1.5 N NaOH was added in a separate volumetric flask and final volume was made to 10 ml with distilled water.

One ml aliquot of the above solution was mixed with 5-ml. alkaline copper tartrate solutions and then incubated for 10 minutes at 40° C and then 1 ml. of Folinphenol reagent was added. After 30 minuted, the absorbance of the solution was read at 600 rim. A calibration curve was also prepared following

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above method and utilizing standard amino acids. The released amino acids were measured through comparisons of assayed and standard curves.

Peroxidase:

The extraction of crude enzyme was carried out as followed for protease activity (Sadasivam and Manickam, 1996).

Assay:

One ml aliquot of enzyme extract prepared earlier and preserved at 4°A was mixed with 7 ml distilled water, 2 ml benzidine solution, 2 ml of 6% H202. The optical density of the solution was measured after 1 minute by spectrophotometer using quartz cuvettes at 610 nrn. The activity measured was expressed in A O.D. (difference) (Mahely and Chance, 1967).

Result

Treatments	Chlorophyll a	Chlorophyll b	Protochlorophyll	a/b ratio
A	**0.43**±0.06	**0.37**±0.04	**0.49**±0.02	**11.06**
B	**0.35**±0.03	**0.27**±0.05	**0.38**±0.02	**1.29**
C	**0.36**±0.02	**0.30**±0.02	**0.42**±0.03	**1.20**
D	**0.32**±0.03	**0.36**±0.05	**0.43**±0.04	**0.88**

Table 1: Chlorophyll content at seedling stage after 7 days of germination as affected by UV-B radiation (3-hr daily) individually and combination of IAA and Kn in *Oryza sativa* crop

Treatment	Chlorophyll	15	30	45	60	75	90
	Chlorophyll a	**0.57**±0.04	**0.64**±0.11	**0.77**±0.06	**1.05**±0.12	**0.96**±0.07	**0.93**±0.07
A	Chlorophyll b	**0.62**±0.03	**0.82**±0.08	**0.96**±0.02	**0.12**±0.08	**0.89**±0.08	**0.80**±0.08
	Protochlorophyll	**0.74**±0.62	**0.92**±0.07	**0.92**±0.13	**0.96**±0.13	**0.96**±0.11	**0.72**±0.02
	a/b ratio	**0.91**	**0.81**	**0.80**	**0.87**	**1.07**	**1.16**
	Chlorophyll a	**0.45**±0.05	**0.62**±0.11	**0.67**±0.06	**0.96**±0.11	**0.92**±0.11	**0.78**±0.05
B	Chlorophyll b	**0.56**±0.03	**0.79**±0.06	**0.89**±0.06	**0.95**±0.05	**0.76**±0.07	**0.65**±0.07
	Protochlorophyll	**0.63**±0.04	**0.87**±0.07	**0.82**±0.06	**0.82**±0.04	**0.75**±0.08	**0.62**±0.05
	a/b ratio	**0.80**	**0.86**	**0.75**	**1.01**	**1.07**	**1.20**
	Chlorophyll a	**0.56**±0.06	**0.65**±0.07	**0.75**±0.06	**0.97**±0.11	**0.87**±0.06	**0.89**±0.11
C	Chlorophyll b	**0.60**±0.07	**0.80**±0.11	**0.87**±0.07	**1.02**±0.06	**0.82**±0.06	**0.82**±0.11
	Protochlorophyll	**0.70**±0.04	**0.89**±0.09	**0.89**±0.11	**0.87**±0.14	**0.92**±0.06	**0.76**±0.09
	a/b ratio	**0.93**	**0.81**	**0.86**	**0.95**	**1.06**	**1.08**
	Chlorophyll a	**0.51**±0.05	**0.66**±0.07	**0.69**±0.11	**1.02**±0.06	**0.95**±0.02	**0.87**±0.06
D	Chlorophyll b	**0.62**±0.06	**0.78**±0.11	**0.90**±0.13	**0.96**±0.06	**0.88**±0.03	**0.67**±0.07
	Protochlorophyll	**0.73**±0.07	**0.87**±0.06	**0.91**±0.66	**0.92**±0.06	**0.95**±0.03	**0.73**±0.006
	a/b ratio	**0.82**	**0.84**	**0.76**	**1.06**	**1.07**	**1.29**

Table 2: Chlorophyll contents as affected by UV-Bradiation (5-daily) individually and combition of IAA and Kn in field grown *Oryza sativa.*

Treatments	*Oryza sativa*
A	**0.23±0.06**
B	**0.61±0.07**
C	**0.36±0.09**
D	**0.46±0.07**

Table 3: Anthocynin as affected by UV-B radiation(3-hr daily) indiidually and in combination of IAA and Kn in field grown *Oryza sativa*

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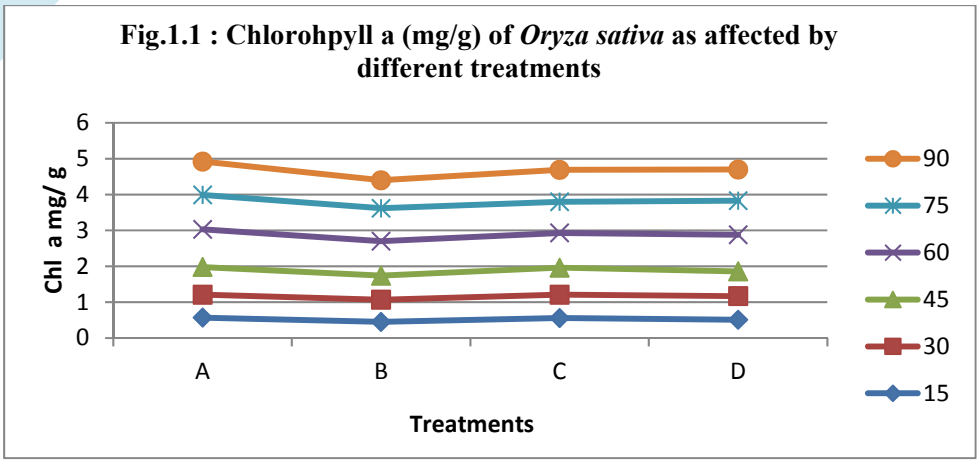

Fig.1.1 : Chlorohpyll a (mg/g) of *Oryza sativa* as affected by different treatments

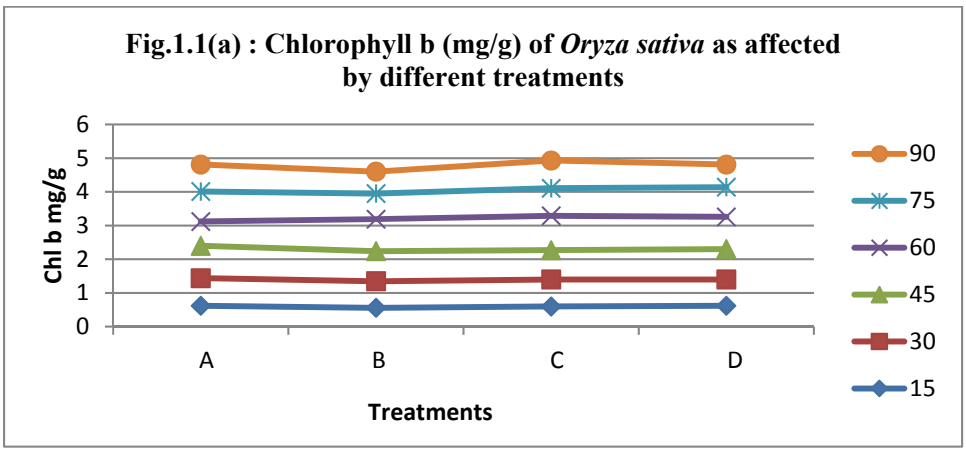

Fig.1.1(a) : Chlorophyll b (mg/g) of *Oryza sativa* as affected by different treatments

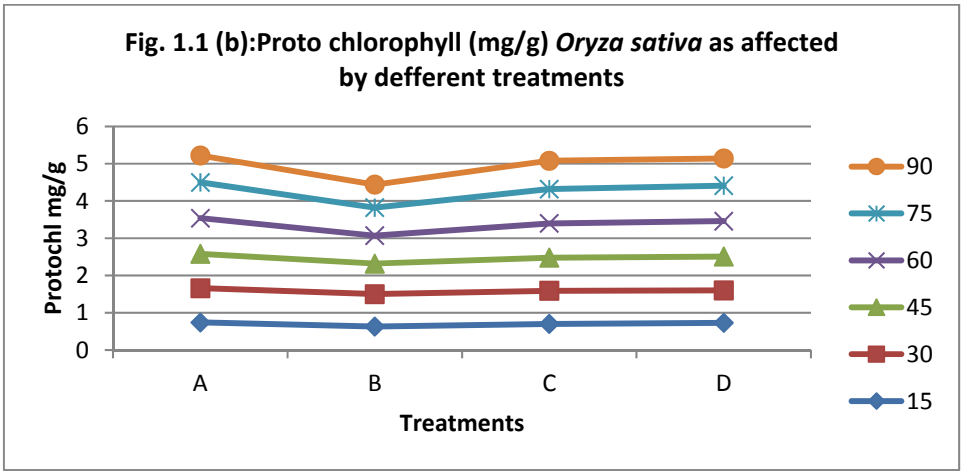

Fig. 1.1 (b):Proto chlorophyll (mg/g) *Oryza sativa* as affected by defferent treatments

Stage	A	B	C	D
Dry	4.200±0.06	-	-	-
6-hr	7.670±0.060	9.067±0.060	10.257±0.70	8.378±0.017
12-hr	8.728±0.070	10.934±0.080	10.537±0.06	10.622±0.02
24-hr	10.288±0.037	16.735±0.43	16.080±0.12	15.689±0.89

Table 4 : Protease activity as affected by UV-B radition (3-hr daily) individually and in combination of IAA and Kn during seed imbibition in *Oryza sativa*

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Stage	A	B	C	D
Dry	4.05±0.07	-	-	-
6-hr	0.425±0.06	0.356±0.05	0.398±0.06	0.387±0.03
12-hr	0.480±0.06	0.687±0.07	0.782±0.09	0.826±0,07
24-hr	0.487±0.07	0.843±0.04	0.927±0.4	0.727±0.072

Table 5 : Peroxidase activity as affected by UV-B radition (3-hr daily) individually and in combination of IAA and Kn during seed imbibition in *Oryza sativa*

Counteracting effects of most of these parameters by different plant growth regulators were also observed during various experiments. These effects are directly or indirectly related to the physiological processes of plants. It can be said that these morphological changes caused by UV-B are the result of physiological distortion. So, in the present study, it is desired to investigate Some physiological parameters in relation to individual UV-B exposure and in combination with certain plant growth regulators.

Chlorophyll Pigment During Seedling Growth

Surface sterilized seeds of Rice was imbibed in water for 6-hr Distilled water washed seeds were transferred to 9 cm petridish (9 diameter cm) for germination and seedling growth studies and treated with UV-B radiation (3-hr daily) alone and alongwith different concentration of plant growth regulators. Chlorophyll a, b and Protochiorophyll were measured after 7 days of growth in both the crops as described in material & methods. The results are presented in table 1.1 for *Oryza sativa*.

A perusal of result in Table 1.1 showed that 3-hr daily UV-B radiation alone and in combination with IAA & Kn affects the different chlorophyll pigments. In control set, the values of these pigments were observed as 0.43±0.06, 0.37±0.04, 0.49±0.02 and 1.16. A marked reduction in chlorophyll pigments except a/b ratio was recorded in plot B, which was subjected to daily 3-hr UV-B exposure. The chlorophyll development was found inhibited by Ca. 19%, 27% and 23% in terms of chlorophyll a, chlorophyll b and protochiorophyll respectively by UV-B treatments. When the seedlings were subjected to PGRs alongwith UV-B treatments, a general promotion was observed in all chlorophyll pigments as compared to UV-B alone. IAA was found to record promotion as Ca. 3%, 11%, 10% in chlorophyll a, chlorophyll b and protochlorophyll and Kn was found Ca. 13%, 13% in terms of chlorophyll b and protochlorophyll. But in case of chlorophyll a, Kn was reported to cause inhibition by ca. 9% as compared to UV-B radiation alone (set B).

Chlorophyll Development During Crop Growth

Effects of UV-B radiation alone and in combination with plant growth regulators on chlorophyll development were also carried out in the s cereals, which were grown earlier for growth pattern studies. Plants for chlorophyll estimation were sampled regularly at 15 days

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interval from seedling emergence up to maturity.

The data set in table 1.2 & fig. 1.2 showed that (3-hr daily) UV-B irradiation alone and in combination of PGRS affected the different chlorophyll pigment in *Oryza sativa*. In control (Plot A), chlorophyll a, chlorophyll b, protochlorophyll and a/b ratio were noticed 0.57±0.04, 0.062±0.03, 0.74±0.04, 0.91 respectively at 15 day stage of growth and found increasing continuously for all the pigments as the crop mature. Plot B indicated a marked reduction in all chlorophyll pigments as compared to control plot. Maximum inhibition in chlorophyll a, chlorophyll b, protochlorophyll and chlorophyll ratio a/b was observed at 15 days, 45 days, 60 days, 75 days stage of growth and inhibited by ca. 21%, Ca. 6%, Ca. 20% and 23% respectively as compared to plot A. The plot C and D reveal promotion of chlorophyll content at all stages of crop growth as compared to individual UV-B treatment (plot B). Kn was observed maximum promotory for different chlorophyll pigment as compared to UV-B treatment alone. The maximum promotion of chlorophyll a, chlorophyll b, protochlorophyll was observed at 75 days stage of growth and recorded as Ca. 15%, Ca. 16%, Ca. 27% respectively as compared to individual UV-B treatment.

Anthocyanins

The effect of UV-B radiation individually and in combination of IAA and Kn on anthocyanin development was studied in rice. Seeds of the crops were presoaked in distilled water in dark for 24 hours and transferred in different petridish for germination and further growth. One petridish carrying seeds of crop was exposed to ordinary white light and treated as control. One petridish of crop was exposedto daily 3-hr UV-B only. Three petridishes of the crops was exposed to UV-B alongwith different plant growth regulators and were carried out in growth chamber. Three days old seedlings were taken for extraction of the anthocyanin as described in material and method.

Ultraviolet—B radiation has positive effect on the accumulation of anthocyanin in *Oryza sativa* seedling. Plant growth regulators viz. IAA (10^{-6}M) and Kn (10^{-6}M) were observed counteracting and lowered down the anthocyanin accumulation induced by UV-B treatment. A perusal three hours daily UV-B exposure caused the marked promotion of anthocyanin pigment in *Oryza sativa.* A promotion of Ca. 165% was recorded in anthocyanin accumulation as compared to control. Different plant growth regulators-viz. IAA and Kn, when given alongwith 3-hr daily UV-B irradiation, were found inhibitory to anthocyanin accumulation level. IAA was recorded much effective to inhibit the accumulation of this pigment and observed Ca. 40% reduction of anthocyanin as compared to UV-B only. Kn showed Ca. 25% reduction as compared to individual UV-B exposure.

Enzymes

Protease:

Effect of UV-B irradiation alone and in combination of plant growth regulators on the protease activity was studied in the seeds of

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Rice. Uniformly selected seeds were soaked in distilled water for 6 hrs, 12 hrs & 24 hrs respectively. Now these presoaked seeds were spread in different petridishes (A, B, C, D). One petridish was kept as control (Neither UV-B nor PGRs), another was exposed to only 3-hr daily UV-B radiation and two petridishes were added with IAA & Kn respectively and exposed to 3- hrs daily UV-B radiation. After providing different treatments, development of protease activity was measured as described in materials & method.

Effect of individual: exposure of UV-B and in combination of PGRs on protease activity in *Oryza sativa* seeds was also studied (table 1.4). After imbibition in water, there was a considerable rise in activity of protease. Data obtained fom petridish A was 7.670±0.060,8.728±0.070,10.288±0.037 at 6 hr, 12 hrs, 24 hrs respectively. Pertridisb B showed an increase by ca. 86% 167% & 87% at 6 hrs, 12 hrs, 24 hrs respectively as compared to control (A). The petridish C & D showed a rise of ca. 104%, 141%, 126& 103%,114%,79% at 6 hrs, 12 hrs, 24 hrs respectively as compared to UV-B treatment alone.

Peroxidase

In order to test the effect of UV-B irradiation individually and in combination of IAA & Kn on peroxides activity, investigations were made on *Oryza sativa* during the course of seed imbibition.

Table 1.5 reveals that peroxidase was linearly increased in case of *Oryza sativa* in individual UV-B treatment. This proxithse activity was reported in control as 0.425±0.06, 0.480±0.06, 0.487±0.07 at 6 hrs, 12 hrs, 24 hrs respectively petridish B studied and recorded an increase of ca. 83%, 143%, 173% at 6. hrs, 12 hrs, 24 hrs respectively as compared to control. Pertidish C & D recorded as increase of Ca. 111%, 113%, 109 and 79% to 32%5 86% at 6 hrs, 12 hrs, 24 hrs respectively as compared to UV-B treatment alone.

Discussion

In The present study, carried out in the laboratory, destruction of chlorophyll, a, b, protochlorophyll and chi a/b ratio was noticed when both the crops were treated with UV-B radiation. The *Oryza sativa* was found more sensitive in chlorophyll reduction. In this crop, chlorophyll a and chlorophyll b were found almost equally reduced due to 3-hr daily treatment of UV-B. When the crops were supplemented with PGRs in addition to the UV-13 radiation, a promotory effect was noted in the present study. Kn (10^{-7}M) was found most promising growth regulator when compared with IAA. Significant reductions in different chlorophyll pigment by UV-B exposure were also investigated by Jain and Goyal (1990), Duysen *et al.* (1985), Sharma *et al.* (1988), Goyal *et al.* (1991), Ambrish (1992) and Dhingra (1999).

The chlorophyll content were also analysed in the field grown crops under the influence of various treatments. In general, it was observed that UV-B inhibits the chlorophyll development throughout the crop age. However, more reduction was recorded in early stages of growth and at maturity. Kn,

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when applied with UV-B radiation, was found to enhance the different chlorophyll pigments level in both the crops, however, the other PGRs also mitigate the adverse effects of UV-B, marginally.

These findings showed the lethal effects of UV-B towards chlorophyll development and repaired by Kn 10^{-6}M. This effect was found variable with the crop skecies. Vu *et al.* (1981, 1983) reported that chlorophyll a/b ratio decreased due to UV-B radiation in soybean but increased in pea.

Tevini *et al.* (1981) concluded that UV-B radiation inhibited the biosynthesis of chlorophyll b than chlorophyll a. Jain and Goyal (1990), while working with lentil crop under field conditions, reported the similar results. They also emphasized that interconversion of protochiorophyll to chlorophyll was retarded. As Kn was found to improve the synthesis of chlorophyll even under increased radiation energy (Purohit, 1988), an improvement in different chlorophyll contents was reported in the present study under similar conditions. One of the measures, which plants develop for the defence towards higher UV-B radiation, is the development of anthocyanin. Present study showed that the crops *Oryza sativa* develop over 165% anthocyanin production as compared to control when treated with 3-hr daily UV-B radiation. A slight decrease in anthocyanin content was noted when the crops were exposed to combined effects of UV-B and different growth regulators. This shows that growth regulators caused insignificant change in the anthocyanin accumulation in plants towards UV-B radiation. Ambler *et al.* (1975) and Bennett (1981) found the accumulation of anthocyanin as a defence of cotton plants against enhanced UV-B radiation. Hashimoto *et al.* (1991) also reported the similar observation, while working with chlorophyll due to enhanced UV-B radiation can be correlated with each other. Enhancement of anthocyanin synthesis can be explained as chloroplast may provide a large reserve pool for the biosynthesis of anthocyanin (Mancinelli *et al.*, 1975). UV-B induced anthocyanins production has also been reported in mustard hypocotyles, corn, wheat and rye coleoptiles (Wellmann, 1982). Arakawa *et al.* (1985) found synergistic increase in anthocyanin production caused by UV-B (312 nm) with white light in apple fruits. Yatsuhashi and Hashimoto (1985) found multi facet action of UV-B photoreepto and phytochrome in the synthesis of anthocyanin using 290 nm (UV-B radiation).

Similar to anthocyanin, flavonoid concentration was also increased, in UV-B treated seedlings after four days of treatment. In contrast, high UV-B fluence increased the flavonoid accumulation (Prem Kumar *et al.,* 2001). According to (Tevini *et al.,* 1990) flavonoid accumulation is regarded as protective mechanism in higher plants to provide against UV-B radiation.

Hence, it is concluded that the UV-B treated seedlings may activate a defense mechanism against UV-B damage by increasing flavonoid. Pal *et al*, (1999) concluded that flavonoid

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concentration can reduce the UV-B penetration and protect the photosynthetic apparatus upto some extent, but it depends upon threshold level which may vary in different species. However, there is also evidence that flavonoids may function in plants to screen harmful radiation, bind phytotoxins and help to regulate the stress response by controlling auxin transport (Shirley, 2002).

This study showed considerable rise in protease and peroxidase activities in the germinating seeds as compared with the pre-existing enzymes in the seeds. Experimental data showed enhancement of protease activity up to Ca. 8% in UV-B exposed germinating seeds as compared to control. When the crop was subjected to combine treatment of PGRs with UV-B, Kn (10^{-6}M) was found most mitigatory which lowered the activity of peroxidase up to 125% while protease activity was lowered slightly when compared to UV-B individual treatment. No significant effects were observed in peroxidase activity with IAA.

Conclusion

1. Experimental studies showed pronounced effect of UV-B exposure and PGRs individually and in, combination on chlorophyll development, of seedlings of the crops. Results of the present study show decrease of Ca. 19%, 27%, 23% for chlorophyll a, chlorophyll b, proto chlorophyll in case *Oryza sativa* respectively after 15 days of seedling growth. When the seedlings were treated with UV-B alongwith IAA it showed as 2%, 11%, 10% for chlorophyll a, chlorophyll b, protochiorophyll. Kn+UV-B showed as 13%, 13%, 9% for chlorophyll a, chlorophylll b, protochiorophyll respectively, when individual treatment of UV-B was given to field grown crops a decline of 21%, 20%, 23% in *Oryza sativa* was recorded for chlorophyl a, chlorophyll b and protochlorophyll. When crops were suppleniented with combination of UV-B and PGRs, an increase in different chlorophyll contents was recorded. Out of the two PGRs, was found to an improved the chlorophyll contents in case of *Oryza sativa*, PGRs were treated with UV-B radiation. Kn was observed maximum promotory for different chlorophyll pigment as compared to UV-B treatment alone. The maximum promotion in chlorophyll a, chlorophyll b and proto chlorophyll was noted as 15%, 16% and 27% respectively.
2. Plants develop anthocynin as a protection pigment against UV-B radiation as evidenced by present as well as other experimental studies. When the seedlings were treated with UV-B, a marked promotion of Ca. 165% of anthocynin pigment was recorded due to UV-B treatment in *Oryza sativa* respectively.
3. The protease activity was also enhanced in germinating seeds of crops due to UV-B radiation. A rise Ca. 86%, 167%, 87% was recorded after 6 hr; 12 hr. 24 hr of soaking in *Oryza sativa* respectively in treated seeds as compared to control.

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4. A marked increase in peroxidase was noted in inhibited seeds of both the crops due to UV-B radiation. An increase of ca. 13%, 96%, 96% *Oryza sativa* after 6 hr, 12 hr and 24 hr of imbibition respectively due to UV-B (3-hr daily) radiation as compared to control.

   All the parameters considered during the present study such as Photosynthetic pigments viz, chlorophyll a, chlorophyll b and protochiorophyll and enzymes Protase as well as Proxidase were also reduced when subjected to UV-B radiation. Effect of UV-B on Wheat and Rice, as far as anthocyanin is concerned was reported an enhancement. It can be assumed after overall studies that accumulation of anthocyanin because of UV-B could act as a screen by absorbing UV-B radiation and in turn protect the chioroplast from UV-B induced damage.

When these most important cereals viz. & Rice were treated with UV-B alongwith PGRS (IAA & Kn), a counteracting effect was reported in all the parameters studied. So, it has been concluded in our study that these plant growth regulators (IAA & Kn) can mitigate the hazardous or deleterious effects caused by UV-B in these cereal crops significantly.

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