CHAPTER I : REVIEW OF LITERATURE

Taxonomic status of *Fagopyrum dibotrys (D.Don) Hara*.

The name *Polygonum cymosum* was mentioned in the Trevian Delect Sem. Hort. Vartisl, 1824; nom nud in Nov Act Acad Caes Leop, which was renamed as *Fagopyrum cymosum* by Meissn in Wall Pl As Rar iii 63; in De Prodr XIV 1 114; Bab .in Trans Linn. Soc XVIII,119; Lindl Bot Reg , 1842 t 26 .Other published names such as *Polygonum* ***cymosum*** (Carol nat. Cur 13: 77 ,1826 . and Steward I C 117 ,1930), *Polygonum* triangulare (Wall Cat ,1689) ; *Polygonum acutatum* (Lehm Cat Sem Hort Hamb ,1820) , *Polygonum emarginatum* (Wall Cat 1688) *Polygonum volubile* (Turea in Bull Soc. Imp Nat Mosq ,1840 ,77 ,) *Polygonum rugosum* (Herb Ham) *Polygonum dibotrys* (D Don , 1825) were given for *Fagopyrum dibotrys* (D Don) Hara .

It has been observed that *Fagopyrum triangulare* (Meissn l .l.c), *Fagopyrum emarginatum var kunawarense* (Meissn in D C l. c 143), *Fagopyrum cymosum* (Trev) Meissn (Hooker, 1886) *Fagopyrum dibotrys* (D Don) Hara (Hara, 1966; Grierson and Long, 1983) were the plant names used by the different taxonomists in their literature.
In some of the books, it has been found that there was no proper author citation in the plant names (Bamber, 1916; Tanaka 1976, 1991; Atkinson, 1980; Kiritikar and Basu, 1975; Singh and Kachroo, 1976). Subba (1982) in his book " Agriculture of Sikkim " mentioned *Fagopyrum cymosum* I as the cultivated plants species.

The taxonomic position of *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Geartn were mentioned by Hooker (1884), Kirtkikar and Basu (1975) as well as Grierson and Long, (1983).

Fagopyrum esculentum was mentioned by Moench in Method 290; Meissn in Wall Pl As Rar iii 63; DC Prodr xiv 1: 143; Bab in Trans Linn Soc xviii 117 Fagopyrum emarginatum Meissn in DC i.c excl var B; Bab i.c 118;

Similarly synonyms were worked out for *Fagopyrum tataricum* mentioned by Gaertn Fruct ii 182, t 119, f 6; Meissn in DC Prodr xiv 144; *F rotundatum* Bab in Trans Linn Soc xviii 117; Meissn l.c *Polygonum tataricum* Linn; Don Prodr 74 1825; Meissn Monog Polyg 62 .t 4. 8; Dala and Gibs Bomb Fl Suppl. 74 .

Medicinal importance of different species of *Fagopyrum*

The earlier mention of the medicinal use of plants were found in the Rigveda. perhaps the oldest repository of human knowledge. having been written between 4500 and 1600 B C. Susruta Samhita which was written not later than 1000 B C contains a comprehensive chapter of therapeutics and Charaka Samihita was written about the description of the materia medica. (Chopra *et. al.*, 1956). From literature survey, it appear that grains of *Fagopyrum cymosum* Meissn was recommended as a diet in colic chloraic , diarrhoea , fluxes and abdominal obstruction (Chopra et al ,1956) .The rhizome of *Fagopyrum dibotrys* (D.Don) Hara was reported for its used against the pulmonary abscess (Chopra et al ,1969). *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn also had the same effects as reported by Samaiya and Saxena, (1986) Chopra et al (1956) and Srivastava and Gupta, (1982). Honey made from buckwheat plant had been observed to be good especially for peptic ulcers. It also contained anti haemorrhagic factors believed to be vitamin K by the Chinese herbal practitioners (Richard, 1977).

Commercial importance of different species of *Fagopyrum*

In India, *Fagopyrum esculentum* is grown almost entirely as a food crop in the temperate parts of the Himalayan ranges and the hilly areas of Tamil Nadu (Singh, 1961). When Couch et al (1940) reported that it was the most promising and economical source of rutin and for which *Fagopyrum esculentum* and *Fagopyrum tataricum* are being cultivated widely in several parts of the world viz., USA, Canada, USSR, Europe, Japan, South Africa, India and Nepal. The rutin content of *Fagopyrum esculentum* 1.9 –4.9 % (Ibanea, et al, 1949; Tang et al, 1989), *F. tataricum* 5.3% (Tang et al, 1989) and *Fagopyrum cymosum* 4-8.5% rutin (Imai and Furuya, 1951) have been worked out though they were not exploited commercially. The seed of *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn contain rutin 1.5-1.56 mg/gm and 8.0-8.12 mg/gm, respectively (Suzuki et al, 1987).

Variation in Rutin content due to environmental condition was studied in *Fagopyrum* species by Ibanez et al, 1949; Naghski et al, 1950; Bassler, 1957 and a number of the papers have been published in connection with the rutin content in *Fagopyrum* species (Schneider et al., 1996; Wagenbreth et al., 1996; Shumnyi, 1985; Singh et al, 1989; Arraiga and Rumbera, 1989; Ilse and Noll 1955; Darwish and Moustafa, 1963; Tang et al., 1989; Suzuki et al., 1987; Ohara et al., 1987; Ohara et al., 1987 Shibata and Yamazaki , 1957 ; Hallop and Margna , 1969).

Chemical constituents in different species of *Fagopyrum*.

The chemical constituents worked out for different species of *Fagopyrum* by different workers from time to time has been tabulated below.

Table 1: Different phenolic compounds reported from *Fagopyrum esculentum* Moench.

Sl no	Plant species	Plant part	Flavonoid / phenolic acid	Reference
1	*Fagopyrum esculentum*	Seedling	Leucoanthocyanin	Troyer (1961)
2	*Fagopyrum esculentum*	Flower	Fagopyrin and Protofagopyrin	Wender , Gortner and Inman (1943)
3	*Fagopyrum sagittatum*	Seedling	Chlorogenic acid and Fagopyrin	Feliks 1968
4	*Fagopyrum esculentum*	Seedling	Rutin	Troyer (1955) Schunck (1858)
5	*Fagopyrum species*	Seedling	Quercitin And Caffeic acid	Hansel and Horhammer (1954)
6	*Fagopyrum emarginatum*	Seedling	Rutin	Grinkerich and I[illegible]va (1970)
7	*Fagopyrum esculentum*	Not specfically mentioned	Quercitin	Zhanaeva,1990
8	*Fagopyrum esculentum*	Not specfically mentioned	Quercitin 3 galactoside	Hansel and Horhammer.1954
9	*Fagopyrum esculentum Moench*	Seed	N5(2hydroxylbenzyl)–all, 4 hydroxyglutamine	Koyama .et al . 1973
10	*Fagopyrum esculentum*	Leaves	Kaempferol 3 rhamno-glucoside p-cumary-lquinic acid and feruloylquinic acid	Krause and Reznik . 1972
11	*Fagopyrum esculentum*	Seed	7-o-_Dglucopyranosyl (1-4)o-D galacto-pyran-oside	Saxena and Sama-iya. 1987)
12	*Fagopyrum esculentum*	Seed	Eriodactyol –5 o methyl ether 7-O D glucopyra-nosyl (1_4) o –pyrano-side	Ibid
13	*Fagopyrum esculentum*	Seed	Taxifolin 3-0 xyloside	Ibid

Table 2: Different phenolic compounds reported from *Fagopyrum dibotrys* (D.Don) Hara

Sl.no .	Name of the species	Plant part	Phenolic Compond	Reference
1	*Fagopyrum dibotrys* (D Don) Hara	Rhizome	Procyanidin B-2	Liang and Xiao , 1990
2	*Fagopyrum dibotrys* (D Don) Hara	Leaves	Rutin	Pradhan and Basu , 1998
3	*Fagopyrum dibotrys* (D Don) Hara	Leaves	Leucoanthocyanin	Basu ,1997
4	*Fagopyrum dibotrys* (D Don) Hara	Leaves	Cyanidin 3 glucoside	Basu and Pradhan , 2000
5	*Fagopyrum dibotrys* (D Don) Hara	Leaves	Cyanidin3,5 diglucoside	Basu , 1997

Table 3a : Different phenolic compounds reported from *Fagopyrum tataricum* Gaertn .

Sl no	Name of the species	Plant part	Triterpenoids	Reference
1	*Fagopyrum tataricum*	seed	Ethyl beta rutinoside	Yasuda and Shinoyama , (1996)
2	*Fagopyrum tataricum*	Leaves and stem	Rutin	Yorash et al (1964)
3	*Fagopyrum tataricum*	Not specifically mentioned	Kaemperol 3 rutino-side	Sato et al ,(1980)
4	*Fagopyrum tatricum*	Not specifically mentioned	quercitin	Underhill et al ,(1964)
5	*Fagopyrum tataricum*	Seed	Kaemperol	Sato et al. ,(1980)

Table 3b: Different phenolic compounds reported from *Fagopyrum cymosum* .

Sl no	Name of the species	Plant part	Phenolic compound	Reference
1	*Fagopyrum cymosum*	Plant	Rutin	Furuya (1952)
2	*Fagopyrum cymosum*	Rhizome	Shakuchirin	Takahashi and Tanaba. (1961)
3	*Fagopyrum cymosum*	Root	5 ·7,3',4' tetrahydro-flavan 3 ol dipolymer	Liu , et al , 1981
4	*Fagopyrum cymosum*	Stem	3 ,4 methylene dioxy 7 hydroxy 6 isopen-tenyl flavone	Samaiya and Saxena (1989)
5	*Fagopyrum cymosum* M	Root&stem	Ferulic acid	Tsuzuki and Yamamato(1989)
6	*Fagopyrum cymosum* M	Root & stem	Caffeic acid	Do
7	*Fagopyrum cymosum* M	Stem &root	Chlorogenic acid	Do
9	*Fagopyrum cymosum*	Rhizome	(-)-epicatechin	Yao et al, (1989)
10	*Fagopyrum cymosum*	Rhizome	3,3' digalloyl procya-nidin	Do
11	*Fagopyrum cymosum* Meissn	Leaves	Quercitin 3 –o-D-(2''–o-p hydroxycouma-royl glupyranoside	Rustogi and Maholatra (1995)
12	*Fagopyrum cymosum*	Dried herb	Hyperin , quercetin and quercitrin	Yamato and Koyama (1962)
13	*Fagopyrum dibotrys* (DDon) Hara	Leaves	Cyanidin 3,5 diglucoside	Basu , 1997

Table 4: Representation of the triterpenoids natural products from *Fagopyrum* species other than *Fagopyrum dibotrys* (D.Don) Hara.

Plants	Plant parts	Triterpenoids	References
Fagopyrum esculentum	Pollen	Brassinolide casterone	Takatsuto et al (1980)
Fagopyrum tataricum	Leaves	P cymene 6.72 % Pinene 8.76% , d – limonene , terpineol , bornyl acetate , thujene, terpenolene mycine	Samaiya and Saxena (1986)
Fagopyrum sagittatum	Not specifically mentioned	ψ-sitosterol	Kaezinave (1968)
F .esculentum Moe-nch	Pollen	Campsterol, stigma-sterol ,ψsitosterol , 24 methylene lencho-lesterol and isofu-sterol	Takatsuto et al , (1990)

Table 5: Representation of the triterpenoids natural products from *Fagopyrum cymosum* or *Fagopyrum cymosum* (Trev) Meissn.

Name of the plants	Plant parts	Triterpenoids	References
Fagopyrum cymosum (Trev) Meisn	Bulb	Hecogenin	Liu et al .,(1983)
Fagopyrum cymosum (Trev) Meisn	Bulb	β-Sitosterol	Liu et al ., (1983)
Fagopyrum cymosum	Not specifically mentioned	β-Sitosterol	Yao et al., (1989)

Table 6: Representation of the triterpenoids natural products from *Fagopyrum dibotrys* (D.Don) Hara

Name of the plants	Plant parts	Triterpenoids	References
Fagopyrum dibotrys (D Don) Hara	Leaves	β-Sitosterol	Basu (1997)

Isolation, purification and characterization of different natural products in different species of *Fagopyrum*.

In general, this plant material is ground or macerated before the extraction. The possibility of enzyme action occurring during the early period of the isolation may lead to hydrolysis of glycosides (Beck, 1964; Trim, 1955). and that can be avoided by plunging the plant materials into boiling solvent or by rapid drying prior to extraction (Seshadri, 1962). Predrying of plant materials generally appear to increase the yield of extraction possibly due to rupture of the cell structure or due to better solvent action (Markham, 1975). Solvents used for the extraction were chosen according to the polarity of chemical constituents to be studied. Extraction with the light petroleum or hexane is frequently carried out to obtain phytosterols. This extraction is also helpful to get rid plant materials of carotenoid, chlorophylls and other fatty substances (Bhutani et al, 1969 a, b, Charke ET al, 1972). Even this treatment may extract certain flavonoid aglycone also. Some methylated flavanone isolated by action of hexane is known to be significantly soluble in light pertroleum (Kupchan ET al, 1969). Hexane is usually used for extraction of nonpolar constituent's extraction (Moriera et al, 1998). Rutin extraction was performed with n-butyl alcohol (Miniati and Montanari, 1998) and ether was used for phenolic acid isolation (Lozovaya, 1999; Gabriele et al 1998).

Flavonoid glycosides and the more polar aglycones, such as flavone, flavanol biflavonyls, aurones and chalchones are generally isolated from plant material by extraction with acetone, alcohol, water or in combination of them (Markham et al, 1970; Ohta and Yagishita, 1970; Bhutanii et al., 1969b; Bhutani et al 1969 a; Cambie and James, 1967). Traces of acid mixed with the solvent were occasionally used for the extraction of flavonoid glycosides (Wallace et al, 1969). Although this practice is normally reserved for the extraction of anthocyanin and anthocyanidins (Seshadri, 1962; Harborne, 1973), the use of acid however, can lead to hydrolysis of glycosidic materials

(Markham et al, 1970). Leucoanthocyanin was isolated by continuous hot percolation of acetone. The combined acetone extracts were concentrated to small volume and continuous extraction with ethylacetate gave a mixture of polyphenols and leucoanthocyanidin (Clark and Katekar, 1960). The extracts were streaked on paper chromatographic sheets (Whatman 3 mm) and developed with 2 % acetic acid (Roux, 1959; Harborne, 1973; Geissman, 1962). The acetone residue was repeatedly extracted with hot water from which the leucoanthocyanidin was extracted from cold anhydrous acetone. The acetone extract was chromatographed with cellulose and developed with water to remove amorphous contaminant from the leucoanthocyanidin (Keppler, 1957). The secondary metabolite , d –catechin was recovered from the ether extract and recrystallized from water (Freudenberg , 1924) . The residue from the ether solution was subjected to recrystallization to obtain l – epicatechin and d – catechin (Freudenberg, 1924) Anthocyanins were extracted from dried plant material with 1-2 % methanolic HCl and subsequently precipitated by the addition of a threefold volume of ether (Hayashi, 1939; Willstatter, 1915).

For the preliminary purification of the crude extract different solvents with varying polarity have been used. This can lead to separation of glycosides from aglycone and to the separation of polar from non- – polar aglycones (Bhutanii et al, 1969 b; Thomas and Mabry, 1968; Margan and Orsler, 1967). Precipitation with lead acetate has been widely used as a method of isolating phenolics (Seshadri, 1962; Seikel, 1962). Recently polyvenyl pyrrolidone (PVP) as precipitant has been suggested for the use in cases when lead acetate is unsatisfactory (Anderson and Sowers, 1968). Treatment of crude plant extract with charcoal powder is also used for the preliminary purification of flavonoids (Mabry et al ,1970). Phenolic acids are considered next to flavonoid as regards their importance in plants (Seikel, 1962). They differ from other phenolic compounds including flavonoids because of their acidic character. Thus phenolic acids are dissolved in dilute bicarbonate and in aqueous sodium acetates. They are soluble in polar solvent, methanol and weakly polar solvent such as ether and ethyl acetate (Seikel, 1962).

Column chromatography remains the single most useful technique for the isolation off large quantity of flavonoids and phenolic acids from crude extract. A technique for separating flavonoid has been discussed by various workers (Seikel, 1962; Mabry et al, 1970; Harborne, 1959 b; Anderman, 1967 a) and the methodology has been well reviewed by Harborne et al., 1975. Absorbant commonly used for the separation of flavonoids include silica gel (Markam et al, 1968 b). magnesol (Ice and Wender , 1962) alumina (Markham and Mabry , 1968 b) cellulose (Seikel , 1962 ; Clark and Porter, . 1972) polyamide (Anderson and Sowers , 1968 , Endres , 1969) sephadex gel (Fischer , 1969 ; Gelotte , 1960) and ion exchage resin (Cassidy , 1957 ; Webster et al , 1967).There are numerous excellent reviews (Kirchnev , 1967 ; Stahl , 1969 ; Truter , 1963 ; Stahl and Mangold , 1967 ; Harborne et al ., 1975 , Harborne , 1973) on the technique used for the separation of phenolic compounds with the help of thin layer chromatography (TLC) . This technique has been developed rapidly during the last few decades and to a limited extent it has replaced paper chromatography (Harborne et al, 1975, Harborne, 1973). It is also complementary to paper chromatography, in that it provides new media for the separation of flavonoids on a small scale and pursuits the use of wider variety of detecting reagents (Markham, 1975). As in column chromatography , the adsorbents of choice for the separation of flavonoids are silica ,polyamide and cellulose . Solvent systems used often vary widely from those used for columns. In general, flavonoids are much more strongly held in thin layer and as a result, solvents of higher polarity are required for their elution (Markham, 1975, Harborne, 1973). Various solvents so far used for the separation of flavonoids and phenolic acids and the spray reagents for detecting these chemical compounds have been reviewed by Harborne et al, 1975 and the aspects of the separation and detection of various sterols have been discussed by Harborne (1973). Besides spectral characteristics of flavonoids, phenolic acid s and sterols have been reviewed by various authors (Jackman, 1959; Cross and Jones, 1969; Scheinmann, 1970; Harborne et al, 1975; Pavia et al, 1979).

Quantitative estimation of phenolic compounds in *Fagopyrum* species.

Bakh (1954) used direct absorptiometry of paper chromatograms for quantitative estimation of flavonoids. Chromatograms of alcoholic extract of flavonoid was developed by ascending technique in the upper phase of a mixture of isoamyl alcohol –petroleum ether – Ac OH -H_2O (3:1:3:3 v/v/v) until the front of the solvent reaches the forty centimeters mark. The paper strips containing the flavonoid were scanned in a spectrophotometer at 350 nm. These method was satisfactory for rutin alone and for buckwheat containing four other flavonoids (Troyer ,1956) . Rutin and quercetin were estimated by Sakamoto and Takamura (1978) by using $SnCl_2.2H_2o$.Quantitative determination by chromatospectrophotometric method after developing the silica gel plates in Et oAc –ACOH – H_2O (10:43:1 v/v/v) was supposed to be the best for separation of the flavonoids using dioxane –water (1:1) with shaking (1 hour) and measured the absorbance measured at 363 nm (Balandiria, 1980). A colorimetric method for quantitative estimation of rutin based on reaction with diazotized sulfanilic acid was worked out (Brejcha, 1958). Russian estimation with the reaction mixture containing (0.1 M Aluminium chloride, 1M potassium acetate and 1N HCl) and allowing to stand at room temperature for about 20 minute measuring at 420 nm had also been used (Sethi, 1997). Some of the recent advances in other plants for the quantitative estimations of polyhydroxyflavones by high performance liquid chromatographic method using a solid phase borate complex has been developed and reversed phase chromatography with diode assay has been used for quick separation of rutin, myricetin, morin, quercetin, fisetin and kaemperol without significant interference of other components, indicating high selectivity (Tsuchiya, 1998). Similarly, analysis of commercial multi-vitamin and rutin by HPLC with diode detector has also developed by Chu (1998). The identification of flavonoid, naphthodianthrone and phloroglucinol constituents was performed using combined HPLC –diode detection (DAD) analysis, HPLC thermospray and HPLC –electrospray mass spectrometry. The flavonoids such as rutin, hypericin, quercetin etc were separated by an aqueous phosphoric acid acetonitrile –methanol gradient within 50 min. The quantification of the above constituents was performed using rutin as an external standard (Brolis, 1998). Conditions for spectrophotometric determination of rutin, by complex formation with Uranil (II) ion, were investigated (Kurtic et al, 1998).

Catechin can be determined quantitatively by means of the colour produced when they are treated with 1-% vanillin in 70% sulphuric acid w/v (Swain and Hillis, 1959).

Leucoanthocyanidins can be determined after heating alcoholic solutions containing butanol: conc. HCl (20: 1 v/v) for forty minutes and estimating the resultant anthocyanidin spectrophotometrically (Harborne, 1964). 5,7,3', 4' tetrahydroxy 3 flavanol was determined after heating with Fe So_4 under acidic condition following the colorimetry by Jund (1986). Quantitative determination of procyanidin B-2 was done using TLC densitometry with the solvent mixture toluence – methyl formate methyl alcohol formic acid (1:2 :0.2 : 0.1 v/v/v) following measurement at 280 nm (Liang and Xiao , 1990). Reversed phase HPLC was used for determination of Rutin in buckwheat grains and flour where a Nvdeocil 7 C_{18}column was used. The eluting solvent contained 2.5 % hooch: MeOH: CH $_3$CN(35:5:10 v/v/v) and absorbance was measured at 350nm (Ohara et al, 1987).

Bioactivity of natural products isolated from different species of *Fagopyrum*.

Among the natural compounds, the flavones are the most important to show therapeutic properties. Dr Szent Gyorgi observed that there were synergistic substances in the food that tended to potentiate the vitamin C activity. When tested, these chemicals were found to decrease the breakage of small blood vessels which ultimately prolonged life in guinea pigs having deficiency in vitamin C. These were observed to overcome small haemorrhages in human subjects who were vitamin C deficient (Armentuno, 1936; Geissman, 1962). This group of substance was called vitamin P, referring to their effect on permeability of small vessels or capillaries. The designation of vitamin P was dropped in 1950 on the recommendation of the American Institute of Nutrition. Since then, the flavones and flavanols have been termed bioflavonoid, which enhance the antiscorbutic activity of ascorbic acid (Zloch. 1909;Bland. 1984). Bland (1984) reported that the decrease in blood cholesterol by bioflavonoid administered with vitamin C. Bioflavonoids has been reported to protect vitamin C, which are transported to the site where vitamin C is to be stored in the cell (Scarborough, 1949). Bioflavonoids and vitamin C being useful in wound healing, in having protective function against various viral infections, incliding oral herpes and in reduction of duodernal ulcer problems, (Bland. 1984). In few clinical studies, bioflavonoids help to reduce the symptoms of menopause, to prevent abnormal platelet adhesion and reduction of inflammation (Robbins, 1980) .The useful activity of bioflavonoid as food supplements, for prevention of retinas hemorrhages. reduction of capillary fragility. reduced risk of stroke of high blood pressure patients, increased protection against arthritis, against rheumatic fever, reduced risk of arteriosclerosis, decreased menopausal symptoms, decreased risk of diabetic cataract, decreased histamine response to allergen exposure. prevention of habitual abortion . reduction of ulcer problems , treatment of dizziness due to inner ear problems , decreased symptoms of asthma , protection against radiation damage and decreased inflammation after injury was mentioned by Shits and Goodhart (1956). A recent review of the flavonoids shows a favorable effect on white blood cells in increasing immune defense, which may account for the anti-inflammatory activity resulting from the oral supplementation (Bland, 1984).

Flavonoids have diuretic activity (Furuya and Ishil, 1972). Flavanone and the flavone glycosides have curative properties to the symptom of scurvy which are not cured by vitamin C (Lockett, 1959). They also confirmed that flavonoid might enhance the synthesis of collagen which was known to be part of the supporting structure of the capillary wall. Fuhrman (1955) reported that the extent of tissue loss in rats with frost bite was less in the group of animals fed with diet containing moderate amounts of flavonoids than in the flavonoid free group, and particularly the flavonoids were more effective in cases of mild injury. Hergert (1962) reported that flavonoids were cardiac stimulants and vaso constrictors and that they increased blood pressure. Flavonoids are also antimicrobial metabolites and accumulate in high concentrations in or around cells damaged by many different stimuli particularly during infection caused by pathogenic fungi and bacteria (Deverall. 1972; Kuc, 1972a).

Rutin is used for the protection against harmful effects of X –rays, cardiovascular system (Alkamatsu, 1931), histamine shock (Wilson, 1947), oral contraceptive (Ryan, 1955), frost bite (Ambrose, 1950), purification of blood, coronary thrombosis, treatment to retintis, prevention of apoplexy (Sood et al, 1982). Armentano et al, (1931) postulated that an additional vitamin is necessary to keep capillary permeability normal. Lovallay and Newmann (1941) suggested that rutin has vitamin P properties. Grifflh et al., (1944) found that it was effective in treating patients with abnormally high blood pressure (Hypertension) complicated by increased capillary fragility. Rutin has been used clinically as an antihaemorrhagic factor in hypertension certain type of hereditary haemorrhagic disorder, hemophilia symptoms, blood poisoning (taxaemia) in pregnancy and as a pre- operative measure to reduce post operative hemorrhage (Atal and Kapur, 1982) Rutin derivative used in brain edema and subpleural hemorrhages has been reported (Benko, 1970).

Rokers and Marti (1951) found that administration of rutin to rats before and after splenectomy reduced the degree of anaemia and leukocytosis. Dried leaves and flower of buckwheat (*Fagopyrum esculentum*) can be used to stabilize permeability and elasticity of the capillary blood vessels thus preventing hemorrhage and ruptures , the main flavonoid and active agent responsible being rutin (Wagenbveth et al . 1996) . Petkov and Manolov (1978) and Schussler et al, (1995) found that rutin have bioactivity to increases coronary flow. Rutin is also effective on the carcinogen induced DNA damage and repair enzymes for protective effect (Webster et al, 1996). It has antimutagenic effects against aflatoxin B1 (Pierzynowska , 1998).

Within the plant, rutin has its effect upon reversing the effect of growth inhibitors, and absicisic acid (Mashtaker et al, 1971; Pradhan and Basu, 1980).

Respiration of uncoupled mitochondria was inhibited by quercetin, a glycone part of rutin (Takahashi, 1998). Sokoray and Czimmer , (1938) studied the action of quercetrin on the blood content of abdominal organs and found that in doses of 1 mg per Kg , there was a fall in the blood pressure. Naghsaki . et al (1947) observed that quercetin possessed considerable toxicity towards *Staphylococcus aureus* completely inhibiting growth at the concentration of 0.1 mg /ml.Quercetin has been observed to inhibit the activity of an aldose reductase (Underhill, 1957) .It has been observed to reduce the hemorrhage in the eye and in reducing the risk of stroke in susceptible patients (Griffith ,1955) . Application of 10 mg, and 20 milligrams per day of quercetin is generally used against to capillary fragillary , easy bruising and small pinpoint hemorrhage called petechiae (Bland , 1984). Kaempferol , hyperin and quercetin -3 methyl ether have also been observed to reduce activity as hypotensives (Laekeman et al , 1986) . Dietary flavonol quercetin induces Cl^- secretion and most likely HCo^{-3} secretion in rat small and large intestine of rat (Cermak et al , 1998) .

Rutin and quercetin pentamethyl ether have been observed to serve as detoxifier of the carcinogen (Wattenberg , 1970) . Dittman et al , (1999) also found that quercetin and its glycosides were weakly inhibitory to glycolysis in human brain tumour slices. Quercetin shows activity against hypertension (Griffith et al . 1955) and hypoglycaemic activity (Dar et al ,1976). Besides this, antioxidant activity has been noted (Heimann et al ,1953; Ashok, et al , 1981).Quercetin , hyperin , rutin , procatechuic acid and 3,4 dihydroxy benzaldehyde are considered as antioxidant compounds (Watanabe et al , 1997) .Quercitin 7 – rhamnoside shows hypotensive activity (Rivera Banilla et al , 1991) and Kaempferol –3 rutinosuide is used as antihypertensive (Ahmad et al , 1993).

Galston (1969) found that monohydroxy phenols such as Kaemperol 3 triglucoside coumaric acid esters and Kaemperol 3 triglycoside are cofactors for IAA oxidase while dihydroxyl phenols such as quercetin 3 triglucosides p coumaric acid esters and quercetin 3 triglucosides are growth inhibitors in *Pisum sativum* . The relative antioxidant activity was related to the major phenolic compounds present including gallic acid ,catechin , myricetin,quercetin , caffeic acid , rutin , epicatechin , cyanidin and malvidin 3 glucoside (Frankel et al , 1995 ; Meyer , et al , 1998 b). Further , individual flavonoids including flavan 3-ol (catechins and procyanidins) anthocyanins , flavonols , and a number of phenolic acids showed strong activity in reducing LDL oxidation *in vitro* (Meyer et al , 1998 a,b) .The lipopolysaccharide induced endothelial cytotoxicity has been observed to be due to catechin , myricetin , quercitin , hesperitin and rutin (Melzig and Loose , 1998).The estrogenic activity is in accordance with the known properties of reputed phytoestrogenic isoflavones , such as diadzein , genistein and formononetin (Knight and Eden , 1995) .

Proanthocyanidins exhibit antimutagenic properties against nitroaromatic compounds (Dauer et al . 1998).5,7 dimethyl ether of leucopelargonidin 3-o –d –L rhammoside and 5,3 ' –dimethyl ether of leucocyanidin 3-o –2 –D galactosyl cellobioside were observed to show possessed insulinogenic action

(Sheeja and Augusti , 1992). Leucoanthocyanin are the substances possessed the property of improving vascular resistance (Tayeav , 1948) . 5,7,3',4' tetrahydroxy 3 flavonol from root of *Fagopyrum cymosum* found to be antimicrobial activities (Liu et al , 1981).The dipolymer of 5,7,3',4' tetrahydroflavan –3 ol dipolymer has exhibited expectorant activity in mice enhanced phagocytic activity of the peritoneal macrophages and antipyretic activity in rabbits (Liu et al , 1981) .Srinivasan et al , (1971) reported its important role in the circulatory system by acting on the aggregation of erythrocytes . Procyanidin has antifungal ,antibacterial and even antiviral activity (Heimann et al , 1953 ; Swain, 1977) .

(-) _ Epicatechin 3 galloyl , -(-) epicatechin , procyanidin and 3,3 ' digalloyl procyanidin isolated from rhizome of *Fagopyrum cymosum* share antitumour activity (Yoa et al , 1989). Catechin , epicatechin and mixed epimers of catechins have the capacity to reduce capillary fragility and permeability (Bukin et al , 1954 ; Lavollay et al , 1943) .It is also reported that they could serve as highly active vasoconstrictors (Haley et al , 1947) .They have been observed to increase resistance of corpuscles to hemolysis (Parrot,1947). However epicatechin and ascorbic acid has been used for vascular fragility correction associated with anaphylaxis (Malkiel et al.,1951) . Epicatechin has antioxidant activity (Husiani et al , 1953 ; Watanabe ,1998). It acted as anticoagulant (Martin and Swayne 1949) and decreased fatality caused by roentgen irradiation disease (Field and Revers, 1949) .It inhibits the activity of testicular hyaluronidase (Parrot and Fasquella (1949) , bovine hyaluronidase activity (Rodney et al , 1950) and choline acetulase (Beiler et al , 1950) .

Anthocyanin has protective action on the vitamin and provide protection against UV radiation (Kano and Miyakashi , 1973;1976; Heintze et al., 1959) Delphimidin slightly inhibited the activity of matrix metalloproteinases , which may have been responsible for the inhibition of tumour cell invasiveness (Nagase et al , 1998). Anthocyanin and leucoanthocyanin have antibacterial action with inhibiting respiration and reproduction at the levels

Chailakhayan (1944) did not observe flowering during any treatment of increased amounts of mineral nutrition to *Fagopyrum esculentum* . Buckwheat showed increase in the yield with the Mo , B , Zn when either form of nitrogen i.e , nitrate or ammonium salt were supplied (Steklova and Shkolnik , 1962; Shustova , 1962).The decrease in yield of leaves was comparatively less in buckwheat when nitrate was replaced by ammonium (Kirkby , 1968) . Tayler and White (1950) reported that in fertile soils supply of N was necessary for good yield of plant . Krause and Reznik (1972) observed that plants grown under P ,N deficiency accumulate flavanols to a higher extent. Repeated utilization of phosphorus and sulphur by buckwheat produced no detectable growth difference (Vlasyuk and Grodzinskii 1955). MCA amine salt (R-CH3) and MCA amine salt (R=Cl) increased the yield from 15.9to 18.6 quintal / hectare and increased the 1000 grain weight from 22.9 to 23.4 g . The former decreased the grain protein content from 15.04 to 15.52% whereas the latter increased it from 15.48 to 16.00% (Derbyshin and Kravels , 1981).

Absorption of water insoluble phosphate by *Fagopyrum esculentum* was observed greatest when grown in sandy soil but least in loamy soil (Chirikov and Malyugin , 1944). Mclachlan , (1976) reported that greatest phosphorus uptake was associated with its ability to acidify the rooting medium . Sulphur does not stimulated growth of buckwheat and neither Cl nor S affected transpiration in buckwheat but in combinations with sand and Cl increased yields of buckwheat (Scialer ,1970). At a constant amount of calcium and potassium in the medium buckwheat the yield was the highest at Ca /K ratio 1:4 . The calcium and potassium contents in buckwheat increased with he increase of these cations in the nutrient medium . The increase of potassium only reduced the uptake of calcium by the plant . The highest nitrogen uptake was found at the calcium /potassium ratio 1:4 . The calcium / potassium ratio did not affect the phosphorus uptake (Peterburgskii and Zhitneva , 1968). The percentage of calcium and the root cation exchange and inhibit larva development (Applebaum et al . 1965). Stigmasterol glucoside has anti inflammatory activity (Ortega et al , 1998).

Effect of light on production of biologically active phenolic compounds in different species of *Fagopyrum* .

Light is one of the most important external environmental factors controlling plant growth and it can be introduced into an intact cell with a minimal pertubation of the biological object (Siegelman , 1964). Troyer (1964) observed that leucoanthocyanin content in the hypocotyls of buckwheat seedling grown in continuous darkness increased until 7th day of growth and he reported that the synthesis of leucoanthocyanin fell in seedlings when anthocyanin was formed following light treatment . Hans and Nes (1957) while working with *Fagopyrum esculentum* found that the light dependent anthocyanin synthesis in seedlings is regulated by the phytochrome system and the high energy reaction . Both the type of synthesis follow independently , yet synergistic action was noticed .(Hans and Nes ,1963). Phytochrome is involved in the photocontrol of anthocyanin from L – phenylalanine (Tokhver , and Myadamyurk , 1984) .Role of the light was studied in the other species of *Fagopyrum* (Tohver et al , 1996 ; Ellis , 1987 ; Margna and Vainjen ,1985 ; Ellis and Degruttola , 1989 ; ; HansMohr and Nes , 1963 ; Margna and Hallop , 1971 ; Tohver and Voskresenskaya , 1969). A pulse far red light (20 w/m^2 for min) following the white light exposure suppressed subsequent anthocyanin formation in the dark . The degree of inhibition decreased with increasing exposure time in white light .On the otherhand if the duration of the latter exceed 24 hour a stimulating effect of the far red pulse was found . It was suggested that dark formation of anthocyanins requires phytochrome P_{730} (Tohver a ,1970). However , blue light promoted anthocyanin accumulation upto 20 w/ m^2 . Under far red light , known to affect via phytochrome , the light saturation for anthocyanin formation was reached at 2 w/ m^2 (Tokhver and Mayadamyruk . 1984). Anthocyanin formation was noted , in the dark with exposure to intermittent and continuos red light (1.3 and 6.8 w/m ^{2}), after preliminary exposure of the seedlings to white light (38 w/m^2) for various periods . Then the anthocyanin content was 5- 10 % in the hypocotyls (Margna et al ; 1971 Ellis , 1987 ; Ellis and Daniel , 1939 ; Ilse and Gertrud,1955). The effect of exogenous L phenylalanine and glyphosate on anthocyanin accumulation in buckwheat seedling grown under blue or far red light was investigated with respect to light intensity level . Blue light promoted anthocyanin accumulation with increasing light intensity (Margna and Hallop , 1971;Tohver and Madamurk , 1984 ; Tohver and Voskresenskaya , 1969). Under blue light the phenylalnine biosynthetic pathway via shikimic acid was suppressed by glyphosate (Tohver , et al,1964). The light dependent anthocyanin synthesis is regulated by the phytochrome system and high energy reaction (Hans and Nes ,1963 Tohver, 1964) . The amount of the anthocyanin formed in darkness and that formed after light exposure for 2-7 hours was proportional to light intensity and period of exposure (Hallop, et al.,1968) .Similar response of anthocyanin formation with light and a subsequent dark period was noted (Troyer ,1964 ; Tohver 1970) .It was suggested that the dark formation of anthocyanins after prolonged light exposure (15hours)could be approximately related to P^{730} deficiency in the seedlings (Hans and Nes , 1963 ; Margna 1985).

Effect of carbohydrate on the production of bioactive phenolic compounds in *Fagopyrum* species

Under constant illumination the supply of sucrose increased the anthocyanin pigment formed in the excised material to a value equal to that in the intact plant. (Tohver , 1970 ; Troyer , 1964). Sucrose , glucose or fructose at a concentration of 1 % inhibit the anthocyanin formation during germination of seed in the sugar solution (Margna et al , 1969; Margna and Otter , 1968) , Na_2 HPo_4 (0.01 M) and K_2 HPo_4 (0.05 M) increased anthocyanin accumulation in buckwheat seedling hypocotyls by7.6 and 6.2% , respectively ,but the value increased upto 11.3 % and 11.1 % respectively . when 2 % sucrose was added with the phosphates (Margna and Margna 1969). The presence of sucrose , glucose , fructose or mannitol in water used for seed germination and in the medium for seedling cultivation reduced the content of anthocyanin . Sugars also inhibited the formation of glycoflavones and rutin in buckwheat seedlings . The stimulating effect of phenylalanine was partly prevented by 0.2 M fructose . sucrose or mannitol (Margna , 1970) .Anthocyanin formation in cotyledons of buckwheat seedling was inhibited by 1 % sucrose , glucose , fructose and mannitol solutions supplied at the time of illumination but the effect was less when placed in the solution before the light treatment . Rutin formation was increased by the same treatment (Margna . et al . 1972). However , the leucoanthocyanuin content did not change when anthocyanin synthesis was increased after adding sucrose to the plant. Thus no simple relation exists between leucoanthocyanin and anthocyanin synthesis in buckwheat seedling hypocotyls (Troyer , 1964).

The etiolated intact buckwheat seedling when exposed to a nutrient solution containing glucose (0.1 M) for 16 hrs in light showed decrease in anthocyanin content in the cotyledons and hypocotyls, while the leucoanthocyanin content did not show any marked change (Margna et . 1971) .The increase of leucoanthocyanin and anthocyanin in seedlings were observed after exposing to glucose (0.2M) for 3-5 minutes (Margna , et al., 1971 ; Margna et al., 1972).

Effect of Chemical factors on the growth of *Fagopyrum* species

Chailakhayan (1944) did not observe flowering during any treatment of increased amounts of mineral nutrition to *Fagopyrum esculentum* . Buckwheat showed increase in the yield with the Mo , B , Zn when either form of nitrogen i.e , nitrate or ammonium salt were supplied (Steklova and Shkolnik , 1962; Shustova , 1962).The decrease in yield of leaves was comparatively less in buckwheat when nitrate was replaced by ammonium (Kirkby , 1968) . Tayler and White (1950) reported that in fertile soils supply of N was necessary for good yield of plant . Krause and Reznik (1972) observed that plants grown under P ,N deficiency accumulate flavanols to a higher extent. Repeated utilization of phosphorus and sulphur by buckwheat produced no detectable growth difference (Vlasyuk and Grodzinskii 1955). MCA amine salt (R-CH_3^-) and MCA amine salt (R=Cl) increased the yield from 15.9to 18.6 quintal / hectare and increased the 1000 grain weight from 22.9 to 23.4 g . The former decreased the grain protein content from 15.04 to 15.52% whereas the latter increased it from 15.48 to 16.00% (Derbyshin and Kravels , 1981).

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The best results of grain production were attained following row wise application of the mixture containing 15% N . 15% $P_2 O_5$ and 14% KNO_3 (Gorpdoil . 1969). Effect of various forms of potassium fertilizers on the rate of nutrients uptake by buckwheat plants was studied by Lakishik. 1967 . The activity of buckwheat roots in taking out P_2O_5 from insoluble phosphates on chernozen and podzolized loam became doubled with KNo_3. (Chirikov and Malyugiri . 1944).Buckwheat was observed to develop best on sulfate forms of K fertilizer (Pchelkin . 1970).An aqueous solution containing 1.0 ppm of 2,4 dichlorophenoxyacetic acid was observed to delay germination and caused abnormalities . At a conc . of 10ppm . 4 chlorophenoxy acetic acid . 2 –methyl –4 chlorophenoxy acetic acid . 2 –methyl –4 chlkorophenoxy acetic acid and 2.4 .5 trichlorophenoxyacetic acid root length was reduced and caused abnormalities to the same extent as 2.4 dichlorophenoxy acetic acid did but isopropyl N phenylcarbonate was less effective when applied to soil 2. 4 dichlorophenoxyacetic acid and 2 .methyl 4 chlorophenoxyacetic acid prevented germination also .According to Shutiva (1962) .the exclusion of Zinc from the nutrient medium during the vegetative period slows down the growth of plants in comparison to that of control . Growing of buckwheat plant in nutrient solution with Zn applied during the flowering phase and especially during the third and fourth stages of development leads to a considerable decrease in the yield by 47.7 % and 45.3 %. respectively . This explained the fact that Zinc is an indispensable element for the formation and development of the seed bud . and that about 75 % of Zinc is accumulated in the seed bud of *Fagopyrum esculentum* (Shutiva . 1962).Effect of cobalt was studied by sprinkling the seed of *Fagopyrum esculentum* with 400 mg /kg of 0.04. 0.06 or 0.08% of $Co(No)_3$ solution and then dried in air . The soil of the experimental plots contained the fine sandy loam with N. P and K added at 30 kg/ ha each . The treatment with Co was found to improve chlorophyll level (Elagin , 1970) and chelating agent (EDDHA) at ¼ the molarity of the aluminium added gave near maximum yield for aluminium tolerant buckwheat . As chelating agent added to the nutrient solution, increased the solubility of aluminium but decreased its toxicity to aluminium sensitive plant (Foy and Brown, 1964) . Krause and Reznik (1972) observed that plants grown under P and N deficiency accumulate flavanols to a high extent than those receiving a complete set of nutrients . .

Nozzolillo (1972) reported that gibberllin acid affected the flowering and fruit production of *Fagopyrum esculentum* when two weeks old buckwheat seedlings , growing under 16 hours day , were treated with 0.01 ml 0.2% gibberllic acid . The flowers were morphologically normal except that the pistils were much reduced in size . The fruit production was greatly reduced. In another instance , Nozzolillo , (1970) reported that Gibberllic acid caused formation of abnormal flower buds in buckwheat and some leaf abnormalities associated with increased dry weight as well as the number of nodes .He again reported that maleic hydrazide caused leaf deformation and inhibited flowering in buckwheat .

The effect of thiamine on germination of buckwheat seeds was low , but flowering increased by 45 % . The average weight of roots was observed to be increased by 14% , stems 23% , leaves 48 % and the yield of seeds by 78% (Bluzmanas and Rancelis , 1961). The effect of kinetin on *Fagopyrum esculentum* was studied by Margna , et al , (1985) and found that Ca^{+2} ions reduced stimulatory effect of kinetin on the accumulation of anthocyanins in the plant .

Application of tissue culture in different species of *Fagopyrum*

The culture of isolated plant parts as callus and the growth of free cell groups in suspensions are pioneering efforts of White (1943) .Hilberandt (1946) and others studied on the physiology of cell and tissue growth and differentiation .Organ redifferentiation can be manipulated in undifferentiated tissue i.e. callus by subjecting it to the interacting influence of plant hormones and nutrient constituents (Murashige and Skoog . 1962) . An organized tissue such as root or stem , could be completely changed to a rapidly proliferating undifferentiated mass of cells (callus) and it cultured on nutrients in the medium containing specific growth hormones such as IAA, NAA , IBA ,2,4 D , Kinetin , Gibberellic acid and BAP . Skoog and Miller (1957)demonstrated that the relative ratio of cytokinin to auxin (IAA) determined the nature of organogenesis in tobacco pith tissue. According to Okazawa et al.,(1967), the organogenesis was generally dependent upon the size of the explant culture .The smaller the explant , the less was the regenerative ability .The larger ones consisting of parenchyma . vascular tissue and cambium shared spontaneous initiation of shoot buds irrespective of the auxin cytokinin concentration (Okazawa et al ,1967). Nutrient media used for the initial proliferation of the tissue played a vital role in inducing embrogenesis . Somatic embryogenesis could be induced only in those suspension cultures which are derived from explants grown on an auxin containing medium (Halperin , 1970) .

The capability of producing callus in *Fagopyrum esculentum* Moench was dependent on explant age and species . The pattern of callus variability in *Fagopyrum esculentum* Moench was wider than that of *F tataricum* (L) Geartn The greatest organogenic ability under (1.5 years) cultivation was observed in densely globular slowly grown callus in *Fagopyrum esculentum* Moench. In the callus lines of *Fagopyrum esculentum* Moench , the abundant formation of buds were successfully propagated and rooted (Ramyantseva et al., 1989). Embryo was isolated from the common buckwheat (*F esculentum* Moench) cultured in the presence of 2,4 dichlorophenoxyacetic acid and kinetin for the first 5 days and later transferred to benzylaminopurine plus IAA medium for the growth . From callus tissues developed from hypocotyls and cotyledons , the three types of tissue were selected in later subcultures (a) the strain of callus tissue that produced buds , (b) embryogenic tissue and (c) unorganised callus tissue , lacking any organogenic capacity .

Pretreatment with 2,4 dichlorophenoxyacetic acid increased the number of explants which gave rise to bud forming embryogenic tissue and the plantlets could be easily obtained by inducing adventitious roots on shoots , but spontaneous root development in somatic embryos was infrequent (Neskovic, 1987).

Takahata (1988) worked with the cultured of immature inflourescence of common buckwheat and the perennial buckwheat on B_5 media supplemented with NAA . 2.4 D and BA at various concentrations . Direct shoot production from the inflorescence of common buckwheat was promoted by the addition of 0-2.0 mg/l, NAA 0-2.0 mg/l, BA optimum 0.2 mg/l and NAA + 1.0 mg/l BA . In perennial buckwheat , direct shoot was induced only on the medium containing 1.0 mg/l NAA and 1.0 mg/l BA in lower frequency as compared to that observed with annual type . In presence of more than 1.0 mg/l 2,4 D or more than 2.0 mg/l NAA , callus was well formed in both the types. Shoots were induced from annual type of buckwheat callus on B_5 medium containing 0.2 mg /litre NAA and 1.0 mg/l BA and lacking plant growth regulators , but not from perennial buckwheat . However, a root system was developed on Murashige and Skoog medium containing 1.0 mg /l BA. When the isolated buckwheat cotyledons form calli in the medium containing high 2,4 D (5 mg /l) and kinetin (0.1% mg/l) induced callus formation .It was found to stimulate cell division in the layer between palisade and spongy parenchyma tissue after 72 hours . Low 2,4 D and low kinetin (0.1 mg/l each) , which stimulated root formation in buckwheat cotyledons . induced divisions primarily in spongy parenchyma cells . High benzylaminopurine (10^{-5} M) and low IAA (10^{-6} M) medium favored bud induction , cell induction , cell divisions to the palisade layer (Neskovia , 1985) .

Thiem et al (1987) reported qualitative TLC analysis of cultured tissue of *Fagopyrum esculentum* .The callus cultures revealed the same flavonols . flavone C – glycosides , anthocyanins , phenolic acids and diathrones as in the field grain specimens ,additional unidentified components were also observed . Substantial differences were noted , however, in a qualitative assay .Rutin content was only 1.1 % as compared to 3.5 % in the field grown plants . Addition of phenylalanine to the culture medium enhanced the growth of the callus and biosynthesis of rutin and polyphenol during *Fagopyrum esculentum* and *Fagopyrum tataricum* tissue culture. However , Moumov et al (1987) found relatively high level of procyanidin in cultured tissue as compared to that in normal plant tissue .

Liquid suspension cultures were established from tissues of buckwheat hypocotyl (Gamborg , 1966) .The secondary metabolites were synthesized depending upon the environmental condition .It was observed in the other plant that the dark grown suspension culture did not form flavonoid pigments , but many of enzymes involved in flavone glycoside biosynthesis were detected(Sutter et al 1972 ; Hahlbrock et al 1971) . It was also noticed that the callus exposed to the light produced more the flavonoids and which were absent in absence of light (Brunetand Ibrahim 1973) . Gregor and Reinert (1972) showed that exposure to blue light increased PAL activity and anthocyanin production but the tissues cultured in the dark showed increased tannin production though the exposure of light caused a several – fold increase in all the monomeric compounds . It also appeared to inhibit polymerisation of the leucoanthocyanin(Constabel 1965 ; Forrest 1969 : Butcher , 1988).

Some ecological features of the Darjeeling and Sikkim Himalayas

In general , Sikkim is viewed as a stupendous stairway leading from the western border of Tibetan plateau down to plains of West Bengal .Sikkim is the most humid place in the whole of the Himalayan range because of its proximity to the Bay of Bengal and direct exposure to the effect of moisture laden southwest monsoon . Rainfall variation along the Tista Valley in Mountainous slope of Sikkim . The whole state is a land of mountain crests . It consists of the tangled series of interlacing ridges , rising range above range to the foot of the wall of high peaks and passes which marks the " abode of snow " and its off-shoots (Risley , 1989) Another more striking features of the state is the peculiar V-shaped valleys with steep and often precipitous slopes . In this mountainous state , the most important single factor that determines the rainfall at a place is the altitude (Bandyopadhyay and Singh , 1998).

It was found that the substantial amount of rainfall i.e 58-76 % of the annual totals received during south-west monsoon was followed by 16-30 % in premonsoon season . The least rainfall occurs during winter about 200 cm , in the extreme south to about 400 cm in the Central of the Valley (Samui , 1994) .

Fog is more frequent during early morning and late afternoon hours of south-west monsoon in Sikkim .Fog in Monsoon is mostly associated with inflow of low clouds but in winter it is mainly due to radiation cooling . Monthly average frequency of fog over Gangtok and Tadong as observed by Samui and Gupta (1994) found highest in the month of July and lowest in November . Frequency of cold waves and severe cold waves over Gangto k was studied by Atri et al .. (1995) who reported that severe cold wave cases found highest in February and lowest in January .

Darjeeling district exhibits a typical monsoon climate . with wet summer and large and dry winter . The condition is brought about by the vapour – laden monsoon wind flowing upward from the Bay of Bengal from May to October . The climate (rainfall , temperature and humidity) varies from one part of the district to another corresponding to variation in altitude and configuration of different areas. Elevation wise , the district is unique in having three district climatic zones ,viz ,tropical , temperature and subalpine vegetation's and bringing about biological diversity (Bhujel,1996).

Four climatic seasons were recognised within the district (1) Monsoon/Rainy season (2) Autumn (3) winter and (4) Summer (spring) .The spring and summer were taken separately .

Monsoon or rainy seasons usually ranging from June to September . is marked by continuous rain , clouds ,fog and Mist .The relative humidity is the highest from June to September in the district .It ranges from 95-100% .The autumn is very short and usually stays from last quarter of September to first half of November .It is marked by clear sky . free from clouds ,dusts and mist .It extends upto the immediate first few weeks of winter .The temperature from December to February , on an average in quite low causing a cold winter . In winter (November to March) the frost accumulate , often in large quantities in the Upper elevations . These regions also experience usual snowfall , mostly in January and sometimes in February and March . The upper mountains such as Sandakphu and Phalut at an elevation of about 3600 m. receive frequent snowfall .Winter is followed by a rather hot summer in the month of April and May especially in the lower hills . In the latter half of February out of the four subdivisions of the district , hailstorms in Kalimpong is more windier than the others (Samanta,1998; Bhujel,1996).

The floral diversity of Sikkim is fascinating because of richness of species and diverse community structure . Approximately 4500 species of flowering plants have been estimated to find representation in this region. As many as 362 species of ferns and fern allies had been reported from Sikkim and Darjeeling (Mehra and Bir , 1964). Hooker (1906) attributed the floristic diversity of the Indian subcontinent " to the immigration of plants from widely different bordering countries ,notably Chinese and Malayan on the East and south ,of oriental . European and African on the North " . In Sikkim many such influences were easily noticed by Singh and Chauhan , 1998) and reported that these elements are purely descriptive and full assessment of floristic elements can be made only after external distribution of all the taxa of Sikkim are worked out and analysed on a non-selective basis .

The forest vegetation of Sikkim can be broadly classified into the tropical forests . subtropical forests . temperature and Alpine forests.(Singh and Chauhan, 1998) and the vegetation of Darjeeling too is classified into the tropical , subtropical , temperature , subtemperate and subalpine forests (Bhujel , 1996) . Besides these, Choudhury (1951) also worked out the forest type of Sikkim as well as the classification of the vegetation of the region that was studied earlier by Gamble (1875) Hooker (1906) , Cowan (1929), Champion (1936) and Kanai (1967) . Hara (1966) too had also classified the forest vegetation of Eastern Himalayas into rain- green deciduous forest , mixed broad-leaved forest, evergreen Oak forests, Rhododendron Coniferous forest , Alpine shrub and Meadows temperate deciduous forest. Daphniphyllum forest , Grassland . He too mentioned that the other cereals were *Zea mays* , *Triticum aesticum, Hordeum vulgare* , *Fagopyrum esculentum* , *Fagopyrum tataricum* and *Eleusine coracana* where *Zea mays* found in lower to middle altitudes and *Triticum* ,*Hordeum* and *Fagopyrum* in middle to upper altitudes .

Tropical forest :

The vegetation and flora of this zone ranging up to 900 m consists mainly of tropical moist deciduous to semi-evergreen species with Sal (*Shorea robusta* Geartn) as a dominant species . These forests occur at low altitude bordering the West Bengal in Teesta and Rangeet Valleys . The characteristic species of this type of forests are *Alstonia nerifolia* D.Don . *A scholaris* (Linn) R.Br , *Bombax ceiba* Linn *Chukrasia tubularis* A Juss, *Duabanga grandiflora* (Roxb .ex-D-C) Walp , *Eugenia kurzii* Duthie , *Ficus* sp , *Mangifera sylvatica* Roxb (Burkhill , 1916 ;Gamble , 1893) The shoots and undershrubs of tropical zone are *Barleria cristata* Linn . *B strigosa* Willd, *Buddleja asiatica* Lour , *Clerodendron* *japonicum* (Thunb) Sweet . *C visosum* Vent etc However . Gamble (1878); Rao and Panigrahi 1961 ; Rao (1963) and Gamble (1875) also worked out with the trees , shrubs and large climbers of Darjeeling district.

Interestingly at some places in dry valleys , e.g at Chitam etc Chir Pine (*Pinus roxburghii* Sarg) forests which are generally found in subtropical to temperate regions elsewhere can also be seen thriving well with associated species like *Shorea robusta* Geartn , *Cycas pectinata* Griff *Ficus oligodon* Miq etc (Singh and Chauhan , 1998).

Subtropical forests :

These forests are confined at elevation from 800 to 1500 m especially along the Teesta and Rangeet rivers and their tributaries . It is mainly mixed forest comprising of following tree species . *Adina cordifolia* (Roxb) Hook.f ex Brandis , *Alongium chinese* (Lour) Harms , *Bischofia javanica* Bl , *Callicarpa arborea* Roxb) Miq *Eurya cerasifolia* (D.Don) Kobuski ,*Fraxinus floribunda* Wall etc (Hooker , 1854 , 1855 ; Biswas and Chopra , 1956) . Besides these , Cowan (1929) also worked out the forests of Kalimpong to account its ecological significance . Climbing species of *Piper* , *Smilax* , *Tetrastigma* , *Cissus* are common in the area . Ferns and fern allies along with species of orchids constitute predominant epiphytic flora of this region . Interestingly . these forests are harbouring rich germplasm of orchids (Sen , 1964 ; Singh and Chauhan . 1998 ; Thomson, 1851 ; King and Pantling , 1898).

Subtemperate forest :

Bhujel (1996) categorised the subtemperate vegetation which ranges from 1200 to 1850 m . The most noticeable character of this vegetation is the absence of profuse mosses and lichens that clad the trees so commonly in temperate region .This type is categorised by the presence of *Engelhardia spicata var acerifolia*, *Viola diffusa* . *Ficus oligodon* , *Indigofera dosua* . *Ardisia macrocarpa* . *Agapetus sikkimensis* etc

Temperate forests :

These types of forests are found between 1500 to 3500 m altitude viz Lachen and Lachung valley . These forests can be mainly classified into Broad leaved forests and coniferous forests depending on the predominance of Dicotyledonous trees or coniferous trees . The main components of broad leaved tree species in Sikkim are *Alnus nepalensis* D.Don , *Acer campbelli* Hook .f and Thoms' , *Betula utilis* D.Don . *Engel hardtia spicata* Bl . *Juglans regia* Linn *Quercus lineata* Bl *Q lanata* Smith etc. Oak forests are characteristic feature of this zone . Shruby vegetation is quite dense in temperature forests and is compose of *Berberis umbellata* Wall *B wallichiana* D.C *Elaegnus umbellata* Thunb . *Fagopyrum dibotrys* (D.Don) Hara (Clarke , 1876 , 1885 ; Gammie , 1892; 1959).

Coniferous forests are common in the *Abies densa* Griff ,*Larix griffithiana* Carr , *Picea spinulosa* (Griff) Henry , *Juniferus* sp . These forests are common in Lachen valley , Zemu valley , Lachung Valley and Yumthang Valley . At some places in North and West Sikkim pure strands of *Taxus wallichiana* Zucc can also be seen . The undergrowth in these forests comprises of *Acer caudatum* Wall , *Berberis allichiana* D.C etc (Singh and Chauhan , 1998 ; Rap , 1963 ; Smith , 1911) .

Sulalpine Vegetation :

The turban belt of Darjeeling district is characterized by the presence of a subalpine vegetation ranging between 3200 m and 3800m . This region has been categorised by some as alpine region (Biswas , 1959 ; Mitra . 1951) and temperate region by others (Gamble . 1875 ; Kanai . 1966) .The upper ridges of the temperate zone of Singalila mountains display a subalpine character with conspicous stunted dwarf plants, a habit principally prevalent in alpine precipitation zones . The presence of typical alpine meadows that runs from Sandakphu (3600m) along the upper ridges to Phalut (3600 m), Gosha (3800 m) , is the characteristic for the zone .The Common plant species observed in this zone are *Abies densa* , *Rhododendron cinnabarinum* , *R fulgens* , *Impatiens kingii* , *Acer caudatum, Primula rotundifolia* , *P sikkimensis* etc (Bhujel , 1996).

Alpine vegetation :

This zone ranges from 3500 to 5000 m . The lower altitudes of this zone support shrubby species of *Rhododendron* , *Berberis* *Cotoneaster* . *Diapensia himalaica* Hook f. and Thoms etc Among the herbaceous flora. species of *Aconitum* . *Arenaria* . *Anaphalis* , *Astragalus* , *Bryocarpum* . *Epilobium* , *Potentilla* , *Primula* etc are common in this zone .

At higher elevation of alpine zone , as in areas above Thangu in Lachen valley and Yomesamdong in Lachen vallley , the vegetation comprises typical alpine moorland type where tree growth is completely arrested . The stunted bushy growth along these slopes consists of tough clumps of *Juniperus squamata* D.Don , *Rhododendron nobile* .D.Don , *R setosum* D.Don ,*Rosa sericea* Lind *Lonicera tomentella* Hook f and Thoms etc (Rao,1964 ;Hooker .1949-51)

Llonakh Valley situated in North –West and Chholamau plains in extreme north –East of the Sikkim have a unique flora of its kind . It has more affinity with Tibetan than Himalaya flora . Among the woody prostrate species , *Berberis* . *Spiraea* , *Lonicera* . *Hippophae* and *Salix* are abounded in the plateau while in the herbaceous species , the *Arenarias* are the most striking with their hemispheric mounds covered with white flowers . Besides *Poterium filforme* Hook .f ,*Primula sikkememsis* Hook . f, *P tibetica* Watt and species of *Delphinium* are also common (Smith and Cave , 1911, 1913 ; Smith ,1963 ; Strearn and Mitula . 1960 ; Thomson , 1851 ; Marquand , 1929 ; Hooker , 1849) .

The flora of Sikkim is very rich both in luxuriance and diversity . .The region abound in a number of primitive taxa viz *Alcimandra* . *Exbuncklandia* . *Houttuynia* . *Magnolia* , *Michelia* (Singh and Chauhan , 1998)

Some of the endemic species of Eastern Himalayan , which are also found in Sikkim, are *Abies dense* Grift, *Agapets incurvata*(Grift) Steum, *A.sikkimensis*. Airyshaw, *Betula utilis* D.Don., *Dipsacus atratus* Hook f and Thoms ex clarke, *Eribotrya hookeriana* Deene.. *Geum macrosepalum* Ludlow *Myricaria albiflora* Grierson and Long etc.(Singh,1994).

Numerous publication on the flora of these region were published by M.J Barkelay (1850) B.N Gosh (1951-57),K M Mathew (1970 , 1981). A Mukherjee (1991) ;U.C Pradhan (1987,1992) . A.P Das (1981. 1985,1987,1995) .

Characteristic features of Soil in the Sikkim and Darjeeling Himalayas

Banerjee and Chakraborty (1977) reported that on passing from hill to foothill in Darjeeling district , the humic acid / fulvic acid ratio also alters . They reported that the hill soil is less than unity but in terai soil it is greater than unity .Similar report was made by Chakraborty and Chakraborty (1980). The later workers also reported that cation exchange capacity of humic acids and fulvic acid s are in general , higher in the hill soil of Darjeeling district . Gupta and Prasad (1983) extracted humic acids from forest soil of Sikkim and repotted the presence of functional groups to the extent of 293,171 and 227 C mol (P^+) /kg for COOH ,OH and C=O groups respectively . Gupta (1984) reported that the content the content of exchangeable Al in C mol (P^+) /kg of Sikkim soil varies from 0to 75 . The soils of higher altitude contains higher percentage of Al^{+3} than that of the lower elevations . Exchangeable Al^{+3} has been found to be negatively correlated with pH.(r= 0.75)

Khera and Pradhan (1976) reported that the acid soils of Sikkim fixed very high proportional of applied phosphorus . They studied the lime requirement for soil of Sikkim based on exchangeable Aluminium and noted pH of soil to became 5.3 . Dhanija et al (1956) studied the soil in the district of Darjeeling and recorded pH as low as 4.5 to 5.0 .

Adhikari et al .,(1986 ; 1983) studied the minerological properties of some hill soils of North Bengal by X ray , data and electrometric titration methods. They reported that quartz; feldspar and mica (Biotite) dominated the sand and silt fraction. Other primary minerals were Tourmaline; garnet, epidote, zincan and hernblende were reported as the dominant clay mineral. Shaklee et al (1965) studied the morphological, physical, chemical and mineralogical properties of two profiles at the foothill soils of Himalayas and found, that associated with chlorite was the dominant mineral.

The total organic matter content in soil generally increases with the increase in altitude. The high organic matter content of high altitude hill soil is mainly due to low temperature and heavy rainfall. The sharp fluctuation in temperature and moisture favours the formation of humus like substances in the region (Chhetri, 1991). Jenny, (1950) stated that the decomposition of organic matter in soil is a biological process and it is accompanied by uptake of oxygen and liberation of carbon dioxide. The result was confirmed with the view of Bint and Roviva (1955) who reported that that the rate of decomposition of humus increases upto a maximum of about 37 ^{0}C. Mukhopadhyay and Banerjee (1985) also reported that the degree of decomposition of organic matter in the Himalayan region gradually decreases with the increase in altitude. The decomposition of humus in the soil may lead to the formation of a number of organic acids much simpler in composition than humic acid resulting in lowering the pH (Mukherjee, 1976).

The percentage of carbon, nitrogen, and hydrogen in humic acid is greater than that in the corresponding fulvic acid in Sikkim (Chhetri, 1991). These features are considered indicative of the simpler structure of fulvic acids (Kononova, 1966). The percentage of carbon in these acids increases on maturing and found as indicative of the increasing degree of condensation of the aromatic rings (Kononova , 1975) .

A negative correlation between available zinc and organic matter in Sikkim soils could be attributed to strong adsorption of Zinc by organic matter in these soils (Chhetri, 1991). Lindsay (1972) stated that organic matter could interact with zinc .