BLACK CUMIN (*NIGELLA SATIVA* L.) – A REVIEW

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Abstract: Black cumin (*Nigella sativa* L., Family: Ranunculaceae) is an annual herb possessing wide range of medicinal uses apart from its commercial significance as a spice yielding plant. Black cumin seeds are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases. Prophet Mohammad (Peace be Upon Him) said: "Use this Black Seed; it has a cure for every disease except death" (Sahih Bukhari). The plant species is also important cytogenetically and may be used as a model plant for better understanding of gene and chromosome relationship. Despite the major advancement of modern medicine in human health-care, it is still intangible and beyond reach to ailing humanity, especially the destitute and therefore in recent years plant based system has been utilized for traditional medicine and phytotherapy. 'Medicinal plants are gift of nature' and black cumin is one such plant with potential uses, which can be explore for safe and effective herbal medicine for human benefit. Considering nearly all essential aspects of the species (synonym(s), common names, origin of the name, distribution, varieties, plant description, floral biology, pollination biology, scanning electron microscopy of seed surfaces, cultivation, economy, diseases, pest, microscopical and powdered characteristics, biochemical constituents, extraction methods of essential oils, therapeutic uses, insecticidal activity, other uses, clinical trials, biosafety, tissue culture and patents), a monograph is prepared on the laid formulation of WHO (World Health Organization) as well as on other significant parameters (cytogenetics and molecular genetics) with the following objectives: to provide an unabridged repository of references regarding the species for its effective and safe utilization as a 'Potential Medicinal Herb'; for creating awareness regarding the use of plant based medicine; understanding economic status, biosafety and patents for regulating herbal medicinal market Nationally and Internationally and exploration of cytogenetical and genetical aspects.

Keywords: Black cumin, Herbal medicine, *Nigella sativa*

INTRODUCTION

Nigella sativa L. (Family: Ranunculaceae; commonly known as Black Cumin) is an annual herb possessing a wide range of medicinal uses[1,2] not withstanding its commercial significance as a spice yielding plant[3]. Black cumin seeds are most revered (Holy herb of the Middle East – Yarnell and Abascal[4]; can heal every disease except death – Islamic prophet Mohammad; stimulates body's energy and helps recovery from fatigue and dispiritedness – The Canon of Medicine, Avicenna; included in the list of natural drugs of 'Tibb-e-Nabavi'; valuable remedy for number of diseases – Unani Tibb system of medicine) medicinally. WHO (World Health Organization) is providing emphasis on the exploration of medicinal plant species for benefit of human care system. Emphasis has been laid mainly on scientific information, on the safety, efficacy, quality control / quality assurance, dosage, toxicity description of the plant species, therapeutic uses, clinical trials, drug interactions amongst other but genetic resources and its induction must also be taken into consideration for significant utilization of a plant species under consideration. Effective utilization of *N. sativa* for therapeutic purposes as well as for trade will vastly depend upon yield (raw plant product- seeds; bioactive compounds- essential oil) and its quality. Existing germplasms may not substantiate the need for future, if not, at present. Therefore, it is of utmost essentiality to raise desirable plant type(s) in *N. sativa* through induced genetic variations and efficient breeding endeavour. Considering nearly all essential aspects of *N. sativa*, a monograph is conducted with the laid formulation of WHO as well as with other significant parameters which will provide unabridged repository of references for present and future researchers who are looking to eugenize the species as a 'potential medicinal herb' for human benefits.

Synonym(s)

Nigella indica Roxb. ex Flem., *Nigella truncata* Viv.[5]

Common names

English: fennel flower, nutmeg flower, Roman coriander, blackseed or black caraway, black sesame; India: Assamese - kaljeera or kolajeera, Bengali - kalo jeeray, Kannada – Krishna Jeerige, Tamil - karum jeerakam, Hindi/Urdu - kalaunji/mangrail; Russian: Chernushka; Hebrew: Ketzakh; Turkish: çörek out; Arabic: habbat al-barakah; Persian: siyâh dâne; Indonesian: jintan hitam; Bosnian: čurekot[6];

French: nigelle de Crète, toute épice; Germany: Schwarzkümmel; Portuguese: cominho-negro; Spanish: ajenuz, arañuel; Swedish: svartkummin[7].

Origin of the name

Originally black cumin was the common name for *Bunium persicum* and later named as *Carum bulbocastanum*, which is now near extinction and slowly *Carum carvi* graduated to the name and due to inability of the species to all over India, later *N. sativa* was adopted from Portuguese or Turkish merchants[6].

Distribution

The species is cultivated and distributed all over India especially in Punjab, Himachal Pradesh, Gangetic plains, Bihar, Bengal, Assam and Maharashtra. Apart from India, the species is also grown in Syria, Lebanon, Israel and South Europe[8] as well as in Bangladesh, Turkey, Middle-East and the Mediterranean basin[9].

Varieties

Following varieties of cultivated Kala-Zira reported with seed yield (g/plant) from Zira and Saffron Research Station, Sangla, district Kinnaur (Himachal Pradesh), India: Rarang (1.7), Pangi (1.4), Stang (2.1), Barang (1.3), Sanji (1.9), Rispa (2.0), Kanam (2.4), Kilba (1.7), Ribba (1.8), Singla (2.3), Telangi (1.4), Thangi (1.9), Lobsang (2.1), Maiber (2.4), Rogi (1.5), Kothi (1.8), Spillow (2.4), Morang (1.7), Purbani (1.8), Sharboo (1.8) and Sunam (1.8)[10]. Variety NRCSS AN 1 to different agrotechniques is also reported[11]. Cheikh-Rouhou *et al.*[12] also reported varieties namely, Tunisian and Iranian.

Plant description

The species is an erect annual herb (Fig. 1) attaining 30.0 cm to 67.6 cm (mean: 52.18 cm ± 4.42) at maturity. Number of primary branches per plant ranges from 4 to 10 (mean: 7.0±0.71); leaf arrangement alternate, leaf phylotaxy 1-2, pinnae of leaves broad, number of pinna per rachis 5-6; total branches per plant 22.5±4.1 (6-48); flower hermaphrodite with determinate flowing patterns, main axis terminate with a solitary flower (Fig. 1), delicate; flower size 2.74 cm × 2.78 cm; color (Fig. 2) - french blue (43/3 – Horticultural Color Chart); flowers without any involucre of bracts, pedunculate; peduncle long, erect; petalloid sepals broad, ovate in a single whorl, 4-6 mostly 5 and characterized by the presence of nectaries; flower fertility 89.89%; stamens in 3 to 4 whorls (Fig. 3), numerous (32 to 66; 49.6±2.7) and shed their pollen as the filament bent outward during male phase; gynoecium 5, completely united follicles, each with a long indehiscent style and composed of variable number of multi ovule carpel, developing into a follicle after pollination; fruit single partially connected to form a capsule like structure (capsule 5 to 45; mean 20.0±3.37; capsule fertility 94.5%) dehiscence through suture; fruits (length – 0.4 to 1.7 cm, mean 1.03 cm ± 0.13; seta per capsule 4 to 8, mean 5.10±0.10) with numerous seeds (59.29±3.2; average seed production/plant - 935±177.9; seed yield – 1.91 gm; seed viability 80% to 90%); seeds ovate, tetrangular, angles sharp, acute, more tapering at the end (Fig. 4), color black (000021 – British Atlas of Colour, 2007); seed size 2.33 mm ± 0.1 × 1.14 mm ± 0.02.

The quantitative data of the species were provided from plants grown in the Experimental garden of Department of Botany, University of Kalyani (West Bengal plains, Nadia, latitude 22°50' to 24°11' N, longitude 88°09' to 88°48' E, elevation 48 feet above sea level, sandy loamy soil, organic carbon 0.76%, soil pH 6.85 – Mandal *et al.*[13]) during the months of November (15th Nov – sowing; 40 cm between rows and 30 cm between plants) as rabi crop and harvested in last week of March or in first week of April[14].

Floral biology

Andersson[15] suggested that increased allocation to perianths leads to reduced allocation to direct component of fitness. Plants both with and without perianths did not differ in fecundity of total flower number. Further, perianthless plants produced heavier seeds with earlier germination dates than the control plants. No detectable effect of perianth removal was noted on seed viability or the fecundity of plants in the progeny generation. High seed mass and germination speed had positive and independent effects on progeny fecundity. The author was of opinion that it is necessary to determine whether large conspicuous perianths enhance the amount of cross pollination and in such case perianth is to be under stabilizing selection, the optimum phenotype being a compromise between pollinator-mediated selection for larger floral displays and trade off with seed size and/or germination speed. The species are capable of setting seed without being cross pollinated, an advantageous feature in seed crop which should be under strong selection for increased seed production. Finally, the author concluded that resource trade-offs with seed mass and time to germination may facilitate evolutionary reductions in flower size.

Pollination biology

Self pollinated; onset of the male stage stamen stand erect, curved outwards one by one, roughly in whorls and strictly reflecting the order of initiation, pollen grains released when anthers reach a horizontal position; male phase initiated a few days before the

stigmas became receptive and lasted for five days; anther receptivity occurred between 8.00 p.m. to 13.00 p.m. for one day only, male and female stages synchronized on the last day of the flowering; weight of pollen 0.064 mg/flower whereas the volume of nectar 0.13 µl[16]; empty anthers curved up; pollinated stigma erect and made an angle of 180º with the ovary; style and anther length nearly equal 1.73 cm; pollinator honey bee, one bee per flower, visited in morning around 7.00 a.m.; high temperature effect fertilization success by affecting stigma receptivity and accelerating ovule degeneration[17].

Scanning electron microscopy of seed surfaces

Datta and Saha[14] studied seed surface ornamentation and found that surfaces were with distinct reticulation marks; reticulation more prominently raised, pentagonal to polygonal, ovoid or irregular in outline; reticulate rows consisting of smaller tuberculate raised cells, cells either uni- or multi seriate or in aggregation along corners or junction; cells of reticulate lines showed shrinkage structure; bound area with variable number of cells (2-5), each cell comparatively larger, penta-, hexa-, polygonal or rounded in outline; lumen floor depressed or shallow glabrous (Figs. 5-10).

Cultivation

In India *N. sativa* is mostly grown once in a year as rabi crop during the months of October (late)–November to March-April in plains; while, rarely in hills in May-June[18].

1. **Area of Cultivation and Production:** Area of cultivation and annual production (source – Comparative Sales Report 2010, VDM Verlag Dr. Muller AG & Co.) were reported to be – India: 6234600 ha, 254000 t; Turkey: 8122010 ha, 689350 t; USA: 16420 ha, 11200 t; UK: 500 ha, 10 to 20 t respectively.
2. **Climate:** Grows well in cool-dry with light snowfall areas to warm-humid areas. Cool and humid weather favors flowering and seed setting[10].
3. **Soil:** Sandy, loam rich in microbial activity is the most suitable soil for cultivation. The sloppy soils of heavy rainfall areas and leveled and well drained soils of moderate rainfall areas are quite suitable for cultivation. Soil pH 7.0 to 7.5 is favorable for cultivation[10].
4. **Preparation of Land:** One ploughing followed by 2-3 harrowing and leveling will be suitable[10].
5. **Method of Sowing:** Seed sowing or by replanting previous year root stocks. Seed sowing is done during October-November by broadcasting (1.5 kg/hectare) or seed drill method or by line sowing keeping space between lines (30, 40 or 50 cm) and at the depth of 2 cm. After 20 days of sowing thinning of the plant to a distance of 20 cm is done.
   Sowing by bulbs (previous year root stock) is possible when soil moisture content of the field is favorable for deep ploughing i.e. neither too wet nor too dry[10].
6. **Manure and Fertilizer:** NPK (5:3:2) is generally applied every year along the side of the planted bulbs[10].
7. **Weed Control:** Frequent weeding reduce weed competition and produce good environmental condition for growth and development. About 3-5 weeding at an interval of 20 to 25 days is recommended by hand hoe or khurpi[10].
8. **Irrigation:** One or two irrigations at flowering and seed formation stage are helpful to increase grain size and oil content[10].
9. **Harvesting:** Black cumin grown as rabi crop in West Bengal Plains are generally harvested late March to first week of April. The crop harvested before shedding at little green stage gives high aromatic oil contents providing good market. Black cumin retains seed viability longer when it is full ripe. It is rather essential that harvesting is done before shedding (shattering of fruits is a major problem) and therefore 2 to 3 or more pickings can be done to avoid loss of seeds due to shattering of the capsules. The harvested crop is dried under sun and threshed by beating with the stick[10].
10. **Post Harvest Management:** *N. sativa* requires extensive labor in collection and harvest as the capsules (fruit) tend to shatter at maturity. Post harvest management of the fruits usually involves their harvest, one by one, by hand and dry storage till natural dehiscence. The mature fruits do not require much attention as they are self-preserving and their essential oil is a great deterrent to fungal attack, insect attack as well as rodent infestation[19].

Shelf life

The seeds of *N. sativa* store well for one year as planting material and as a spice, they are stored in airtight conditions to prevent the loss of aroma. As a spice, it is recommended to be stored away from other species as the species has a overbearing flavour and aroma and disturb the flavour of other species[10].

Economy

Reports

1. Rs. 275-300/kg in local market (Pakisthan-Mingora, Din, Peshawar, Pindi, Lahore, Gilgit and Astore), whereas in down country it cost Rs. 450-500. In Internatinal market it is sold for Rs. 850-1000/gm[20].

2. Germany: Black cumin oil 1000 ml – 23.90 EUR + shipping cost[21].
3. Black cumin MGS Heirloom Seeds; Product code: NIG02:300 seeds – 1.90€[22].
4. Black cumin USA. (i) Product No. 1130.F, 29.57 millilit. - $1.40. (ii) Product No 1130.G, 59.14 millilit. - $1.99. (iii) Product No. 1130.H, 118.29 millilit. - $2.46. (iv) Product No. 1130.I, 236.58 millilit. - $3.50. (v) Product No. 1130.J, 476.16 millilit. - $6.00. (vi) Product No 1130.K, 5lbs minimum - $5.00. (vii) Product No. 1130.N, 10lbs min. - $4.50. (viii) Product No. 1130.O, 25lbs min. - $3.95[23].
5. Black Seed 100 capsules - $9
   Black Cumin Tea (Organic) 20 Bag - $6[24].
6. In Indian market Rs. 250-300/kg. Since its cultivation fetches high income per unit area, therefore, it is highly suitable for cultivation by marginal farmers[10].

Diseases

Sinha and Singh[25] reported *Macrophomina phaseolina* infection in roots causing its deformation. Wilt (causal organism grows along the seedling and leaves and branches look light green in colour, leaves shed and plant dries up; control: spray Dithane M-45 0.2% or Dithane Z-78 or Blitox 5 w.p. at 15 days interval) and rotting of bulbs (emit a special odour; control: dipping bulbs in 0.3% bavistin for 30 mins. before planting, field kept free from stagnant water) are diseases reported[10]. Early report by McRae and Shaw[26] also suggested *Fusarium* wilt in the species. Prolonged survival of *F. udum* for upto 8 years was reported in roots.

Pest

1. Caterpillar – Makes holes in the bulbs and cut down seedlings.
   Control: Dust the soil at the sowing or hoeing with 5% Aldrine, 10% BHC at the rate of 25 kg per hectare; application of well-decomposed farmyard manure[10].
2. Armyworm and semi-looper – Feed on the flowers, seeds, and damage the crop.
   Control: Spray with 0.05% methyl parathion – 1 ml/l water or Thiodian or Endosal 35EC, 1 ml/l of water at 15 days interval[10].

Microscopical and powdered characteristics

Transverse section of seed show single layered epidermis, thick walled cells, covered externally by a papillose cuticle and dark brown contents; 2-4 layered, thick, tangentially elongated parenchymatous cells followed by reddish brown thick walled rectangular cells; endosperm thin walled, cells rectangular to polygonal filled with oil globules; powdered characteristics brownish black, parenchymatous cells and oil globules[27,28].

Biochemical constituents

Constituents of *N. sativa* seeds are fixed oil – 32 to 40% (saturated fatty acids- about 30%; palmitic acid, stearic and myristic acid; unsaturated fatty acids: arachidonic, eicosadienoic – 3%, linoleic – 50 to 60%; oleic acid – 20%; dihomolinoleic fatty acids – 10%), volatile oil- 0.4 to 0.45% (nigellone, thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citrlnellol, p-cymene), proteins 16-19.9% (arginine, glutamic acid, leucine, lysine, methionine, tyrosine, proline, threonine), minerals 1.79-3.74% (calcium, phosphorus, potassium, sodium, iron), carbohydrate 33.9%, fibre 5.50% and water 6.0%[29]. Ramadan and Morsel[30] reported that apart from physical constants: 2% w/w, foreign matter; 6% w/w, total ash; 0.2% w/w, acid insoluble ash; 20% w/w, alcohol soluble extractive; 15% w/w, water soluble extractive; 3.91% w/w organic matter; 4% w/w, loss on drying[31]. The seeds contain carotene, which is converted to vitamin A in liver[32]. Acetylated triterpene saponin (penta hydroxyl pentocyclic triterpene) has been isolated from the species[33].

Phytochemical Compounds: Categorically different phytochemical compounds of seeds are nigellone[34], nigellicine, nigellimine, nigellimine-N-oxide, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, 24-ethyl-lophenol, gramisterol, lophenol, 243-methyllophenol, obtusifoliol, sitosterol, stigmastanol, stigmasterol, stigmasterol-7-ene, beta-amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-O-[α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol[31,35,36], nigellidine[37], carvone, d-limonene, cymene, α, β-unsaturated hydroxy ketone, steroids, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidal saponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methylolean-12-enoate, stigma-5,22-dien-3-β-D-glucopyranoside, cycloart-23-methyl-7,20, 22-triene-3β,25-diol, nigellidine-4-O-sulfite[38], nigellamines A3, A4, A5, C[39], nigellamines A1, A2, B1, and B2[40].

Seed Oil: The seed oil contains cholesterol, campesterol, stigmasterol, β-sitosterol, α-spinasterol, (+)-citronellol, (+)-limonene, p-cymene, citronellyl acetate, carvone[41], nigellone, arachidic, linolenic,

linoleic, myristic, oleic, palmitic, palmitoleic and stearic acids. Fixed oil: linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%). Volatile oil: trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%)[42], 2-(2-methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol[43]. Root and shoot are reported to contain vanillic acid[44].

Extraction methods of essential oil

1. Conventional method – extraction by hexane in Soxhlet[45].
2. Enzymatic extraction[46].
3. Ultrasound assisted extraction[47].
4. Microwaves assisted extraction[48].
5. Supercritical solvent extraction[49].
6. Surfactant assisted method; based on the use of aqueous solution polyethylene glycol sorbitan monolaurate (Tween 20)[50] amongst other methods.

Oil extracted were analyzed and characterized by using classical analytical procedures, spectroscopic and chromatographic methods.

Therapeutic uses

Traditional Uses: In traditional system of medicine black cumin seeds are effective against cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrheal, obesity, diabetes, paralysis, hemiplegia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea[51]. It has also been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic and carminative[52] as well as it is applied to abscesses, nasal ulcers, orchitis, eczema and swollen joints[51]. Seed oil is considered to be local anesthetic[53,54].

Pharmacological Significance: The species possesses antimicrobial (diethyl ether extract and methanol and chlorophyll extract and plant extract as well as seed oil were found to inhibit *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and a pathogenic yeast *Candida albicans* – Hanafy and Hatem[55], Hosseinzadeh *et al.*[56], Chaieb *et al.*[57], Khalid *et al.*[58]), anti-malarial[59], antioxidant (thymoquinine constituent of seed oil, enhance the oxidant scavenging system – Salem[60]), anti-inflammatory (the oil and thymoquinone – Salem[60]; thymoquinone has the ability to attenuate allergic airway inflammation by inhibiting Th_2 cytokines and eosinophil infiltration into the airways and exploratory effects – Isik *et al.*[61]), anticancerous (methanolic extract of plant exhibits potent inhibition of cancerous cell growth against HL-60 and U-937 cell lines with IC_{50} value 13.50 µg/ml and 28.31 µg/ml respectively – Raval *et al.*[62]), antitumerogenic (active components – thymoquinone and dithymoquinone; tymoquinone kill cancer cell by a process that involved apoptosis and cell cycle arrest with little effect in non-cancerous cells – Buyukozturk *et al.*[63]), anti-hypertensive[64], antiviral (Infections Laryngotrachietis virus – Zaher *et al.*[65]), anti-asthmatic (crude seed extracts exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade – Kalus *et al.*[66]), anti-allergic (oil is an important adjuvant for the treatment of allergic disease – Dahri *et al.*[67]), anti-diabetic, antilipidemic, antiobesity[9], anticonvulsant[43,68], antitoxic[69] properties apart from having immunomodulatory (extract inhibit human neutrophil elastase activity which is mainly attributed to carvacrol – Mansi[70]), hematological (oil play role in modulating the balance of fibrinolysis/thrombus formation by modulating the fibrinolytic potential of endothelial cells – Gilani *et al.*[71], Zaoui *et al.*[72]), gastro-protective (thymoquinone protect gastric mucosa against injurious effect of absolute alcohol and promote ulcer healing – Naz[9]), nephroprotective[73,74,75], diuretic[76], cardiovascular (active ingredient thymol has shown to lower blood pressure through blockade of calcium channels - Gilani *et al.*[71], Paarakh[8]) properties as well as the species is protective against heavy metal[77,78], effects nitric acid production[79], possesses analgesic activity (volatile oil – Ramadhan *et al.*[80]) amongst others. Moreover, essential oil was found to be effective against Cr(VI) hazard and may be a promising candidate against different environmental pollutants[81,82] reported that the species is a good absorbent for the removal of cationic metals coming from wastewater. Tasawar *et al.*[82] reported that black cumin (tested on 80 subjects, divided randomly into 2 groups) is effective to change the lipid profile significantly in a way which is beneficial to heart.

Black seed has also been used externally where it is applied directly to abscesses, nasal ulcers, orchitis, eczema and swollen joints[51]. *N. sativa* is also a potential source for antidermaphytic drugs. The ether extract of seeds and its active principle thymoquinone are found to be effective after clinical trials against many species of three important genera of dermatophytes: *Trichophyton*, *Epidemophyton* and *Microsporum*[83,84]. The volatile oil inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contraction induced oxytocin suggesting its anti-oxytocic potential[69]. Hot water extract of NS as well as whole seeds in large oral doses causes abortion in human pregnant females[85]. The species is also used in long term treatment of opioid defense[86]. Thymoquinone has been reported to exhibit effect on dopaminergic neurons against Parkinson's disease[87].

Insecticidal activity

Essential oil from dried fruits was isolated by hydrodistillation and tested for its repellent, toxic and developmental inhibitory activities against wheat

flour pest *Tribolium castaneum*[88]. Results indicated that the essential oil reduced the oviposition potential and increased the developmental period of *T. castaneum* in comparison to control group. Fumigation of essential oil inhibited development of larvae to pupae and the pupae to adults and also resulted in the deformities in the different developmental stages of the insects. All the responses were found concentration-dependent.

Other uses

N. sativa seed cakes in the feed of buffalo and lambs improved their body weight and reproductivity as well as seeds in the food of broiler chicks improved their immunity and feed conversion efficacy[89,90].

Clinical trials

Significance of the species has been documented from some clinical trial experiments. Al-Ghamdi[91] administered aqueous suspension of the seeds orally at two dose levels (250 mg/kg and 500 mg/kg) for five days to assess carbon tetrachloride (CCl_4)-induced liver damage. CCl_4 (250 microl/kg intraperitonelly/day in olive oil) was given to the experimental group on days 4 and 5, while the control group was only treated with the vehicles. Animals treated with CCl_4 showed remarkable centrilobular fatty changes and moderate inflammatory infiltrate in the form of neutrophil and mononuclear cells when compared to the controls. This effect was significantly decreased in animals pretreated with *N. sativa*. Histopathological or biochemical changes were not evident following administration of *N. sativa* alone. Serum levels of aspartic transaminase (AST), L-alanine aminotransferase (ALT) were slightly decreased while lactate dehydrogenase (LDH) was significantly increased in animals treated with CCl_4 when compared to control group. LDH was restored to normal but ALT and AST levels were increased in animals pretreated with *N. sativa*. In conclusion, it appeared that seeds are possible safe and protective against CCl_4-induced hepatoxicity.

Ali and Blunden[92] examined the hypolipidemic and antioxidant effects of dietary black seed in hyperlipidemic rabbits (24 male rabbits were fed with 0.5% cholesterol diet for 1 month, randomly assigned to two groups – control group received the hypercholesterolemic diet and the black seed group was fed 7.5 g/kg b.w/day crushed black seed + 0.5% cholesterol diet, each for 2 months). Fasting blood samples were obtained at baseline, after hyperlipidemia, 1 month and 2 months of treatment to determine serum lipid profile, malondialdehyde (MDA) level, total antioxidant status (TAS), superoxide dismutase (SOD) and glutathione peroxidase (GPX). Results indicated that black seed can favorably decrease serum lipid profile and lipid peroxidation levels in hyperlipidemic rabbits, thereby indicating that seeds may be considered as a useful therapy for hyperlipidemia.

Abbas *et al.*[93] reported that *N. sativa* oil possesses anti-inflammatory and bronchodilator activities. Clinical trial with mouse model suggested that *N. sativa* significantly reduced blood eosinophil count; IgG1 and IgG2a levels, cytokine profiles and inflammatory cells in lung tissue. These effects were comparable to the effects of dexamethasone except unchanged IFN-y level.

Abou-Gabal *et al.*[94] studied the effect of the oral administration of aqueous suspension of *N. sativa* (50 mg/kg.b.wt) against chromosomal aberrations and ultrastructural changes of the bone marrow cells in mice treated with carbon tetrachloride CCl_4 (two dose level: 1.9 ml/kg.b.wt and 3.8 ml/kg.b.wt). Mitotic activity decreased in bone marrow cells of animals treated with CCl_4 as well as significant increase in the number of bone marrow cells with different types of chromosomal aberrations was recorded. Ultrastructural changes were also dose-dependent including both nucleus and cytoplasm of erythroid and myeloid elements of the bone marrow cells. Treatment of the animals with *N. sativa* improved both genotoxicity and ultrastructural changes induced by CCl_4.

Al-Kubaisy and Al-Noaemi[95] reported protective role of seed oil against effect of CCl_4 on the liver cells.

Samir Bashandy[96] reported that administration of NS oil to hyperlipidemic rats improved their reproductive efficiency (increase in seminal vesicle weight, testosterone level, sperm motility and sperm count and a decrease in sperm abnormalities) and produced additional protection against hyperlipidemia induced reduction in fertility.

Najmi *et al.*[97] performed clinical study (2 groups of 30 patients each) to evaluate the adjuvant effect of seed oil on various clinical and biochemical parameters of the metabolic syndrome. Group I (standard group) patients were given Atorvastatin 10 mg once a day and tablet Metformin 500 mg twice a day along with *N. sativa* seed oil 2.5 ml twice a day for six weeks. Results indicated that Group III patients showed significant improvement with reference to total cholesterol, low density lipoprotein and fasting blood glucose, thereby indicating that seed oil is effective as an add-on therapy in patients with metabolic syndrome and also possessing therapeutic activity in diabetic and dyslipidemic patients.

Al-Sa′aidi *et al.*[98] determine the effect of alcoholic extract of black seed *N. sativa* on fertility parameters in white rat. A total of 60 mature males were divided into 3 groups – the first group (control) intake drinking water, while the other two groups (T_1 and T_2) intake the extract in two doses (0.5 and 1.5 g/kg respectively) daily for 53 days. The results revealed that treatment with alcoholic extract of *N. sativa* led to significant increase (P<0.01) in body weight gain

(g), reproductive parameters (seminiferous tubules thickness and diameters, account of spermatogonia, primary and secondary spermatocytes, spermatids, free spermatozoa, account of sertoli and Leydig cells, diameter of Leydig cells and the height of epithelial cells entirely covered epididymal caudal), hormones (testosterone and follicle stimulating hormone) as well as protein concentration and significant decrease (P<0.01) in leutinizing hormone and cholesterol concentration.

Mohammad *et al.*[99] from clinical trial experiments with male albino rats suggested that the aqueous extracts of *N. sativa* have increased spermatogenesis activity in seminiferous tubule.

Al-Attar and Al-Taisan[100] reported the preventive effects of black cumin seeds (seed extract – 300 mg/kg/day) on Spragu Dawley Rats (clinical trial performed with 50 male rats, divided into four groups) exposed to Diazinon. Results indicated that seeds can be considered therapeutic agent against hematotoxicity, immunotoxicity, hepatoxicity, nephrotoxicity and cardiotoxicity induced by diazinon and may be against other chemical pollutants, environmental contaminants and pathogenic factors.

El-Naggar[101] investigated the cytotoxicity of *N. sativa* dry methanolic extract on cultured cortical neurons and its influence on neurotransmitter release, as well as the presence of excitatory (glutamate and aspartate) and inhibitory amino acids (gamma-aminobutyric acid-GABA- and glycine). The secretion of different amino acids was studied in primary cultured cortical neurons by HPLC using a derivation before injection with dansyl chloride. NS modulated amino acid release in cultured neurons; GABA was significantly increased whereas secretion of glutamate, aspartate, and glycine were decreased.

Mohamed *et al.*[102] investigated protective role of *N. sativa* in DAB (dimethylaminoazobenzene) induced liver carcinogenesis. The study included 140 Albino mice weighing 40-50 gm divided into 4 groups: Group I - normal control; Group II - *N. sativa* treated control; Group III – treated with DAB; Group IV – treated with *N. sativa* and DAB. Biochemical investigations, flow cytometric analysis and histopathological examination of the liver tissue were performed and the results showed significant change in the DNA content, histomorphology, and antioxidant enzymes in liver tissues of the DAB treated group. These changes were restored to normal with *N. sativa* treatment. Further, it was noted that treatment with *N. sativa* only showed comparable result with control untreated group. Thus, it was inferred that *N. sativa* lonely induce no harmful effect on the liver rather it exerts hepatoprotective effect against liver carcinogens.

Al-Naqeep *et al.*[103] reported (experiment conducted on HC rabbit) that *N. sativa* seeds powder or oil showed hypocholesterolemic and antiatherogenic cardioprotective properties.

Attia *et al.*[104] performed experiment on male rats and were of opinion that omega-3 polysaturated fatty acid (ω_3) and seed oil of *N. sativa* might prevent oxidative stress and attenuate the changes in the biochemical parameters (levels of urea, creatine, total bilirubin and uric acid contents and aminotransferase, phosphatases, and lactic dehydrogenase) induced by Lindane (r-HCH-r-hexachlorocyclohexane).

El-Gohary *et al.*[105] studied the effect of carboplatin (a synthetic antineoplastic agent used for cancer treatment) and *N. sativa* oil alone or in combination on human breast cancer cell (MCF-7) *in vitro* and Ehrlich as cites tumor bearing female mice (*in vivo*). The *in vitro* experiment on MCF-7 cells illustrated that IC_{50} of carboplatin was 11.8 µg/ml, IC_{50} of *N. sativa* oil was 39 µg/ml and IC_{50} of the combination between carboplatin and black cumin oil was 3.78 and 40 µg/ml respectively. The *in vivo* experiment illustrated that carboplatin (10 mg/kg) increased the enzyme activity of aspartate amino transferase (GOT) and aniline amino transferase (GPT) by 56.52% and 51.14% respectively as compared to both healthy control (non-tumor transplanted mice) and negative control. The activity of GOT and GPT was increased by 14.75% and 19.84% respectively as compared to healthy control under the effect of *N. sativa* oil (12 ml/kg); while, the enzyme activities decreased in comparison to negative control. The combination of carboplatin and oil appeared to increase the enzyme activity of GOT and GPT by 62.41% and 49.39% respectively compared to both healthy control and negative control. Agarose gel electrophoresis revealed that carboplatin induced DNA damage of liver tissue but *N. sativa* oil showed intact DNA without any damage.

Parhizkar *et al.*[106] studied the estrogenic activity of *N. sativa* by vaginal cornification assay using an ovariectomized rat model (40 ovariectomized Sprague Dawley rats, weighing 250 to 350 g were used; NS powder given at 300, 600 and 1200 mg/kg for 21 consecutive days; compared with 0.2 mg/kg conjugated Equine estrogen as positive control). Data obtained from vaginal smear suggested that NS possesses estrogenic function which can be helpful in managing menopausal symptoms as an alternative for Hormone Replacement Therapy.

Rayan *et al.*[107] studied the effect of black cumin oil (BSO) against *Toxoplasma gondii* Me 49 strain in a murine model of infection. After clinical diagnosis with mice (35 mice were studied in 3 groups) and assessment of survival rate and brain cyst burden, brain histopathological lesions and immunohistochemical expression of inducible nitric oxide synthase (iNOS) it was noted that BSO in prophylactic or therapeutic regimens significantly enhanced protection of infected mice against death (P=0.01) and reduced brain cyst burdens at 5, 7 and 12 weeks post infection compared to the infected untreated control.

Antitumor- Ait *et al.*[108] suggested that essential oil (IC_{50}=0.6% v/v) and ethyl acetate (IC_{50}=0.75%) extracts were more cytotoxic against P8-15 cell line than butanol extract (IC_{50}=2%). The authors further suggested that TQ induced apoptosis and inhibited proliferation in pancreatic ductal adenocarcinoma cells. TQ also increased P^{21}WAF1 expression, inhibited histone deacetylase activity and induced histone hyperacetylation. TQ is reported that it acts as a novel inhibitor of pro-inflammatory pathways which combines anti-inflammatory and proapoptotic modes of action. Banerjee *et al.*[109] performed *in vitro* studies on pancreatic cancer cells preexposed with thymoquinone (25 µmol/l) for 48 h followed by gemcitabine or oxaliplatin resulted in 60 to 80% growth inhibition compared with 15 to 25% when gemcitabine or thymoquinone was used alone which suggested that the mechanism of thymoquinone could potentiate the killing of pancreatic cancer cells by down regulation of nuclear factor kappa B (NF-kappa B), Bcl-2 family, and NF-kappa B-dependent antiapoptotic genes. Breyer *et al.*[110] tested 4-acylhydrazones and 6-alkyl derivatives of thymoquinone for growth inhibition of human HL-60, leukemia, 518A2 melanoma, KB-VI/Vbl cervix and MCF-7/Topo breast carcinoma cells. The 6-hencosahexaenyl conjugate was most active in all resistant tumor cells, with IC_{50} (72 h) values as low as 30 Nm in MCF-7/Topo cells. Nagi and Almakki[111] investigated the effect of thymoquinone (TQ) *in vivo* and *in vitro* male albino rats on fibrosarcoma induced by 20-methylcholanthrene. It was found to inhibit tumor incidence and tumor burden significantly. Shafi *et al.*[112] reported methanol (IC_{50}-2.28 µg/ml), n-hexane (IC_{50}-2.20 µg/ml) and chloroform (IC_{50}-0.41 µg/ml) extracts of the seeds effectively killed HeLa cells by inducing apoptosis.

Diabetic and Cardiovascular Activities- Meddah *et al.*[113] observed improvement of glucose tolerance and body weight in rats after chronic oral administration *in vivo*, which validate the traditional use of black cumin seeds against diabetes. Chandra *et al.*[114] reported that HIV protease inhibitors, nelfinavir (5-10 µM), saquinavir (5-10 µM) and atazanavir (5-20 µM) with *N. sativa* seed extract decreases glucose stimulated insulin secretion from rat pancreatic beta-cells. Altan *et al.*[115] were of opinion that combined treatment with NS and hPTH alone in improving bone mass, connectivity, biomechanical behavior and strength in insulin-dependent diabetic rats. NS treatment alone or in combinations significantly increased the area of insulin immunoreactive beta-cells in diabetic rats suggesting that NS might be useful in the treatment of diabetic osteopenia. Kanter *et al.*[116] and Kaleem *et al.*[117] suggested that oral administration of ethanol extract of black cumin seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improvement altered levels of lipid peroxidation products and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney. Meral *et al.*[118] suggested that NS might be used in diabetic patients to prevent lipid peroxidation, increase in anti-oxidant defense system activity and also to prevent liver damage. al-Awadi *et al.*[119] reported the significance of NS seeds for its use in non-insulin dependent diabetic mellitus. An aqueous decoction of a plant mixture containing NS was found to lower blood glucose level after oral administration[120]. Al-Hader *et al.*[121] suggested that intraperitoneal administration of volatile oil of seeds produced a significant hypoglycemic effect in normal and alloxan induced diabetic rabbit.

Oral supplement of *N. sativa* seeds to normal rats was investigated and the results showed intrinsic cardiac properties without evidence of an increased cardiac work load or energy consumption *in vivo* which makes the seeds an isotropic agent with hemodynamic profile[77,122,123]. Shafei *et al.*[124] examined the effects of aqueous and macerated extracts from *N. sativa* on heart rate and contractility of the isolated heart. Results showed a potent inhibitory effect of both extracts on both heart rate and contractility of guinea pig heart that was comparable and even higher than that of diltazem which may be due to calcium channel inhibitory or an opening effect for the plant on potassium channels of the isolated heart. Dichloromethane extract of seeds (0.6 ml/kg/day), essential oil and unsaponifiable matter of oil, volatile oil and thymoquinone found to be cardioprotective[125,126,76,67]. Gilani *et al.*[127] reported that thymol has shown lower blood pressure through blockade of calcium channels. The effect of oral treatment of Wister albino rats with different doses of powdered seeds (100, 200, 400 and 600 mg/kg/day) for four weeks on the levels of serum lipid was investigated, and it was found that it causes significant decrease in low density lipoprotein-cholesterol levels, triglyceride levels and increase in high density lipoprotein-cholesterol level[128].

Pulmonary Activity- Nigellone was found to inhibit effectively the histamine release from the mast cells suggesting its use in asthma[129]. Padmalatha *et al.*[130] studied the antinaphylactic effect of a polyherbal formulation containing NS on mesenteric mast cells. The antinaphylactic activity was possibly due to the membrane stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release. Gilani *et al.*[127] suggested that bronchodilatory effect of NS seeds was mediated possibly through calcium channel blockade. Keyhanmanesh *et al.*[131] studied the prophylactic effect of TQ on tracheal responsiveness and WBC (white blood cell) count in lung lavage of sensitized guinea pigs. The results suggested the preventive effect of TQ on tracheal responsiveness and inflammatory cells of lung lavage of sensitized

guinea pigs. Suddek[132] was of opinion that TQ-induced relaxation of the precontracted pulmonary artery is probably by the activation of ATP-sensitive potassium channels and possibly by non-competitive blocking of serotonin, alpha-I and endothelin receptors.

Immunomodulation- Islam *et al.*[133] studied the effect of volatile oil of *N. sativa* seeds (NSVO) for its immunomodulating and cytotoxic properties in rats and it was found that there was a significant decrease in splenocyte and neutrophil counts, but a rise in peripheral lymphocytes and monocytes in rats. LC_{50} values for NSVO were 155.02±10.4, 185.77±2.9, 120.40±20.5, 384.53±12.1 and 286.83±23.3 micro g/ml respectively against the SCL, SCL-6, SCL-376, NUGC-4 cancer lines and 3T6 fibroblast line. Results indicate NSVO as a potential immunosuppressive cytotoxic agent. Swamy and Tan[134] performed *in vitro* cytotoxicity of seed extracts (in ethyl acetate fraction) in different cancer cell lines P388, Molt 4, Wehi 164, LL/2, HePG2, SW 620 and J82 as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the ethyl-acetate column chromatographic fraction (CC-5) showed selectivity against HePG2, Molt 4 and LL/2. CC-5 was relatively non-toxic against human umbilical cord endothelial cells at 50 µg/ml. Results therefore indicated that CC-5 possesses a potent cytotoxic effect as well as a potentiating effect on the cellular immune response.

Contraceptive Activity- Hexane extract of the seeds prevented pregnancy in Sprague-Dawley rats treated orally at 2 g/kg daily dose on day's 1-10 post – coitum. The active hexane extract exhibited only mild euterotrophic activity comparable to ethinyl-estradiol, but was devoid of any estrogenicity in the immature rat bio-assay[135]. Agarwal *et al.*[136] reported that ethanolic extract of seeds possesses antifertility effect in male rats which is probably due to inherent esterogenic activity.

Nephroprotective Activity- Ali[137] investigated the effect of oil (oral treatment: 0.5, 1.0 or 2.0 ml/kg/day for 10 days) on gentamycin induced nephrotoxicity in rats. A dose-dependant amelioration of the biochemical and histological indices of GM nephrotoxicity that was statistically significant at the two higher doses. Treatments enhanced antioxidant status in plasma and also reduced glutathione concentrations in renal cortex and enhanced growth. Badary *et al.*[138] studied the effect of TQ on the nephropathy and oxidative stress induced by doxorubicin (DOX) in rats (10 mg/kg/day – supplemented with drinking water for 5 days before DOX and daily thereafter) and found that TG, TC and serum urea lowered significantly. TQ has been suggested to be protective agent for protienuria and hyperlipidemia associated with nephritic syndrome.

Effectiveness- Qidwai *et al.*[139] performed clinical trial experiment (study design was randomized, double-blind trial) to assess effectiveness, safety, and tolerability of powdered *N. sativa* seeds in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults (123 patients were recruited; 64 and 59 patients were randomized to the intervention and the control arms respectively; 39 patients in the intervention group and 34 in the control group completed the study). Favourable impact of powdered *N. sativa* seed in capsule was noted on almost all variables; however, larger study with adequate sample size was recommended.

Biosafety

1. Seed powder did not produce any toxic effects at very high doses (28 gm/kg orally) in rabbits[140].
2. Seed oil safe when given orally to rats (LD_{50} of 28.8 ml/kg)[72].
3. Oral thymoquinone was found safe (LD_{50} of 2.4 g/kg)[141].
4. Oral thymoquinone (LD_{50} of around 1000mg/kg) and intraperitoneal (LD_{50} of around 100 mg/kg) in mice/rat, safest[142].

Cytological and cytogenetical studies

Karyomorphology: Gregory[143] was pioneer to enumerate the number of chromosomes (2*n*=12) in somatic complement of *N. sativa*. Bhattacharyaya[144] revealed five pairs of very long (L_1) to long (L) chromosomes with median to sub median primary constrictions and a single pair of medium-sized (M) chromosomes with sub-terminal primary constrictions in the species. Secondary constrictions were located in two of the long pairs of chromosomes and karyotype formula was suggested as $2n=12=2L_1+4L^S+4L+2M$.

Saha and Datta[145] reported four morphologically distinct chromosome types (A, B, C, D) in *N. Sativa* (2*n*=12) on the basis of chromosome length (very long 15.0 to 20 µm; long 10.0 to 14.9 µm; median 5.0 to 9.9 µm), nature of primary constriction and presence or absence of secondary constriction (Figs. 11-12). The somatic complement possessed one pair AA (very long, 19.13 µm; F% 44.01), one pair BB (very long, 16.70 µm; both primary- F% 44.88 and secondary constriction were present), three pairs CC (C_1C_1- very long, 15.31 µm; C_2C_2- long, 14.86 µm and C_3C_3- long, 13.80 µm; F% 44.04 to 45.42) and one pair DD (medium 6.64 µm, F% 7.23) chromosomes (TF% 41.38, haploid chromatin length 86.50 µm ± 3.3). The somatic chromosome types could easily be marked in meiotic plates (Figs. 13-14).

Ghosh and Datta[146] karyotyped *N. sativa* through Image Analyzing System (Micro Image ™ Lite Software, Version 4.0 for windows, 47N40155 2000 0515 MAN VG MIX) and revealed four (2*n*=12=4A+4B+2C+2D; karyotype formula: $2L^{sc}_{1sm}+2L_{1m}+2L_{sm}+2S_t$) morphologically distinct chromosome types (L_1= very long≥15.0 µm, L= long

13.0 to <15.0 µm, M= medium 7.0 to <13.0 µm, S= short <7.0 µm; m= metacentric, sm= sub-metacentric, t= telocentric and sc= satellites). The somatic chromosome complements in the species formed graded karyotype which was symmetric in nature (TF% 42.90). Total haploid chromatin length was noted to be 78.62 µm ± 2.87.

Meiotic Analysis and Pollen Fertility: Saha and Datta[145] reported regular 6 bivalents formation at diplotene and metaphase I (MI) in most PMCs (Figs. 15-17); while, the rest demonstrated 5II+2I formations (175 meiocytes assessed). Frequency of bivalent and univalent per cell varied from 5.88 to 6.0 and 0.00 to 0.23 respectively. Frequency of bivalent and univalent per cell was 5.95 and 0.10 respectively. The bivalents formed rings (range- 2.85±0.51 to 3.58±0.24/cell) and rods (range- 2.41±0.24 to 3.15±0.51/cell). Average frequency of ring and rod per cell over the plant was 3.18 and 2.77 respectively. Chiasma per nucleus range between 8.87±0.21 and 9.62±0.32 (average: 9.34±0.28). Frequency of bivalents, ring and rod configurations per cell and chiasma per nucleus showed random distribution over the plants ($p>0.05$) but univalents per cell was non-random ($p<0.01$) as evidenced from χ^2 test of heterogeneity. Mostly (99.49%- pooled over the plants) anaphase I (AI) cells manifested equal 6/6 separation (Fig. 18) of chromosomes, rare often unequal separation (5/7), lagging chromosome and bridges were also noted. Pollen fertility among black cumin plants varied from 95.2% to 100.0% (average: 98.06%). Saha and Datta[145] were further of opinion that the meiotic chromosomes could easily be identified and marked in meiotic plates.

Pachytene Chromosome Analysis: Datta[147] reported that the length of pachytene chromosomes (Fig. 19) in the species ranged from 51.86 µm to 140.55 µm with mostly median primary constrictions (F%: 41.60 to 47.56; arm ratio: 0.71 to 0.91). A telocentric (F%: 12.36; arm ratio: 0.41) was also marked in the pachytene complement. Four (chromosome type A- 140.55 µm; type B- 109.75 µm and 97.89 µm; type C- 94.93 µm and 89.32 µm; type D- 51.86 µm) morphological types were suggested with 2 bivalents (B type) documenting secondary constrictions. However, further studies on the somatic complement has suggested that one pair of chromosome were with secondary constriction[145,146].

Accessory Nucleoli: Rang and Datta[148] revealed consistent presence of single nucleolus (size 8.36 µm ± 0.08) in PMCs (pollen mother cells) of *N. sativa* and it is in accordance to the number of chromosome with secondary constriction in the complement[145,14,146]; however, nucleolus is not commensurable to the number of secondarily constricted chromosomes and it has been proven that those chromosomal regions which code for 18S and 24S RNA are nucleolar organizing in nature[149].

Rang and Datta[148] found 1 (48.39% to 65.57% PMCs; size: 8.30 µm ± 0.18) to 5 (size variation between 1.67 µm and 8.30 µm) nucleoli (Figs. 20-25) in different mutant (1-2: *Lax branching* and *viridis*; 1-5: *bushy*, *chloroxantha*, *crinkle leaf*, *feathery leaf*, *narrow leaf*) lines of *N. sativa*. Nucleoli was either free or found in association to different bivalents but occasionally two nucleoli of different or same sizes were seen attached to a single bivalent. Multiple and variable sized nucleoli formation were presumed as an outcome of disturbed genetic state of the plant types caused by gene mutation and the mutant genes possibly have induced changes in the regulatory system of the cell thereby activating various latent loci capable of synthesizing tiny nucleoli. Hiko-Lchi and Chen-Hui Kao[150] attributed size variation of nucleolus on the basis of difference in the intensity of nucleolar forming power.

Mitotic and Meiotic Abnormalities Arising out of Irradiations: Kumar and Nizam[151] assessed the effect of X-rays on dry and pre-soaked seeds of *N. sativa* and noted that the frequency of mitotic and meiotic aberrations in the pre-soaked seeds was higher than that of the dry seeds. The aberrations encountered were mostly related to spindle organization and formation of dicentrics, rings, micronuclei and acentric fragments. Mandal and Basu[152] studied X-ray induced chromosomal aberration from leaf meristems, pollen mother cells and endosperm and reported that aberration percentage increased with an increase in doses and decrease with time lapse from 2 to 24 hours after irradiations. Most resistant tissue was endosperm though it had the largest Interphase Chromosome Volume (ICV).

Datta and Biswas[153] (X-irradiations to dry seeds, doses- 6, 8, 10, 20, 30 kR, LD_{50}- lie between 8 kR and 10 kR), Datta *et al.*[154] (gamma irradiations- 5, 10, 20, 30, 40, 50 and 60 kR doses, seed moisture- 1.8%, LD_{50}- lie between 20 kR and 30 kR, treatments beyond 30 kR were lethal) and Mukherjee and Datta[155] (gamma irradiations- 50, 100, 150 and 200 Grey, moisture content- 19.04%, LD_{50} lie between 50 Gy and 100 Gy) reported physiological (germination and seedling growth under petriplate conditions) and chromosomal disturbances (mitotic and meiotic including pollen fertility) in irradiated samples. Frequency of total mitotic anomalies enhanced in treatments but the percentage of dividing cells decreased with an increase in the radiation doses, and it was suggested that mitotic disturbances have affected physiological processes like germination and seedling growth. Apart from normal chromosome configuration $2n=12$ (Fig. 26), irradiations (X-irradiation as well as gamma irradiations) have induced chromosomal aberrations like fragments, ring configuration of chromosome, pseudochiasma like configurations, diplochromosomes, cells with polyploid and aneuploid chromosome number and deformed cellular configurations at metaphase (Figs. 27-31), and bridges (single, double, criss-cross, inter-

locked and incomplete) with or without fragments (2 to 4 identical sized and rare often with one fragment- Figs. 32-41), and multipolar organization of chromosomes at anaphase (Figs. 42-43). At resting cells micronuclei (1-4 variable sizes; condensed as well as uncondensed) and giant cells were also noted (Figs. 44-47). Meiotic abnormalities studied following irradiations (apart from normal 6II formation- Figs. 48-49) were univalents (2-8, Figs. 50-52), fragments (paired identical sized- Fig. 53), multivalents (Figs. 54-57), stickiness (Fig. 58) and cell fusion (Fig. 59) at metaphase I (MI); while, fragments, bridges with or without an accompanying fragment were observed in anaphase I and II cells irrespective of normal segregation of chromosome at AI[153,154,155] (Figs. 60-65). Mukherjee and Datta[155] noted enhanced frequency of quadrivalents (mostly ring- 89.79%, rest were of chain configuration) was noted in higher doses of treatments. Most of the ring quadrivalents were of adjacent orientation (63.64%); while, the rest were alternate (34.09%) and rare often non co-oriented (2.27%). A PMC at 200 Gy was observed to posses 6II + two nearly identical sized (2.93 µm and 2.59 µm) fragments (1.21%) thereby suggesting localized breakage in chromosome due to irradiation. Paired identical sized fragments (5.38 µm) at AI was also studied in one of the two telocentric chromosomes (one telocentric is marked intact at one pole). Pollen sterility and meiotic anomalies studied have shown dose dependent increasing tendencies thereby indicating that former is an outcome of the latter.

Rang and Datta[156] exposed dry, pre-soaked (12 hours in distilled water), totally dehydrated and stored (one year six months stored under desiccation; one season stored seed) seed samples (moisture content: 7.5%) of *N. sativa* to gamma irradiations (5, 10 and 20 kR doses) and also that some amount of the dry irradiated materials were treated with ethyl methane sulfonate (EMS) and hydrogen peroxide (H_2O_2) for six hours at 0.25 percent to evaluate the cytogenetic changes that might occur due to gamma-irradiation influenced by the physical and chemical factors. Assessment of radio-sensitivity has been made from attributes like seed germination, rate of seedling growth, mitotic index, frequency and spectrum of chromosomal aberrations in root tip cells and pollen and seed sterilities of M_1 plants as well as M_2 mutation (macromutants) frequency. Results indicated that the factors (physical and combined treatments) have influenced gamma radiation sensitivity in inducing cytogenetical and genetical changes along with M_2 mutation frequency.

Mitotic Abnormalities Induced by Chemical Treatments: Biswas and Bhattacharyaya[157] studied the effect of some mutagenic chemicals like mateic hydrazide (MH), acridine orange (AO), ethyl urethane and ethylene-diamine-tetracetic acid (EDTA) at variable concentrations and durations on the root tip mitosis of the species. The chemicals induced cytological aberrations viz., fragments, laggards, micronuclei, grouping and stickiness of chromosomes and reduced mitotic index in prolonged treatments and in higher concentrations. The authors were of opinion that the chemicals possibly affect nucleic acid synthesis in differential manner which ultimately causes hazards in replication thereby inducing chromosome breakage.

Kumar and Nizam[158] studied induced somatic pairing of homologous chromosomes from root tip mitosis following treatment with mitomycin C. It was observed that the homologous chromosomes become juxtaposed to each other with remarkable regularity in the prometaphase cells following treatment for 40 minutes, whereas the untreated cells showed no such associations. It was presumed that these movements may be due to kinetochore activity which normally causes congregation of chromosomes towards the equatorial plate of the spindle but which does not occur contemporaneously in all chromosomes. In view of the observation, the authors were inclined to believe that kinetochores were responsible for placing homologues near each other and stickiness has been attributed to be a factor for association of homologous chromosomes.

Chand[159] reported that pentachlorophenol (PCP) inhibited mitosis in shorter duration of treatments and cytological abnormalities were formed. Incorporation studies revealed that PCP inhibited DNA synthesis. The chemical was found to affect nuclear membrane cycle, chromosome division cycle, spindle organization and chromosome movement, condensation and spiralization of chromosomes and DNA and protein synthesis.

Induced mutagenesis

Variants in M_1 Generation: Datta and Biswas[160] reported that as compared to the erect nature of the stem in untreated control plants, stem anomalies including bifurcation (Fig. 66), trifurcation (Fig. 67-68), twisting (Fig. 69), unbranched (Fig. 70) and twining nature of stem (Fig. 67) were observed at 4, 10 and 30 kR of X-ray doses and 2 and 4 hours treatment with 0.75% and 0.50% EMS respectively. Interesting floral anomalies were found to occur in all treated doses of EMS and only 20 kR X-irradiation. In relation to control flower (Fig. 71) interesting floral variations like adnation of sepals, elongated and strap shaped petals, two gynoecium in the same flower and presence of bract like structures (incompletely forked) similar to that of the petalloid sepals were observed (Figs. 72-78). The abnormalities studied at M_1 have not recurred in M_2 generation and these were non-inheritable changes (chimeric in nature) possible arising out of somatic mutation.

Macromutants and Their Inheritance Pattern: Kumar and Nizam[161] induced (X-rays and gamma rays) few viable mutants such as multicolor capsular

fruits and color fruit coat with ornamentations including mutation affecting branching pattern and fertility at M_2. Datta and Biswas[160] induced (X-ray and EMS) several chlorophyll (*albina* > *xantha* > *chlorotica* > *chloroxantha* > *albescens* > *albino-terminals* > *xantha-terminals* > *lutea* > *viridis* = *marginata* = *coeruleovirens*) and morphological (13 different types; 9 viable – *lax branching* - Fig. 80, *feathery leaf, bushy, male sterile, crumpled leaf, dwarf, early flowering, prostrate* - Fig. 81, and *brown seed coat*; 4 non-viable types– *cup* - Fig. 85, *needle leaf, crinckle leaf* and *cotyledonary leaf*) mutants in relation to normal trait (Fig. 79). Threshold doses were effective and efficient and 0.5% EMS, 2 hours treatment was the best among all the treated doses. Chlorophyll mutatuions occurred predominantly than other types and among them *viridis* and *chloroxantha* were the viable types and were found to be controlled by two pairs of recessive genes; while, the mutant trait(s) of *bushy*, *dwarf*, *feathery leaf*, *lax branching* and *early flowering* mutants were controlled by a single pair of recessive gene. Datta and Biswas[162] assessed different mutants (*lax branching, feathery leaf, bushy, early flowering, prostrate, dwarf, brown seed coat* and *viridis*) for different quantitative traits at M_2, M_3 and M_4 generations (ANOVA performed in mutant lines with control at M_4) and were of opinion that the mutants have exhibited superiority over the control plants in some of the characters only but not in all the parameters. This observation was significant as it offered scope of improvement through hybridization and selection.

Mitra and Bhowmick[163] induced ten different types of chlorophyll mutation in two cultivars of *N. sativa* following treatments with gamma irradiation and EMS. Higher doses of gamma-rays and lower concentration and duration of EMS were reported to be most efficient. Mitra and Bhoumick[164] studied the mutagenic effects (gamma irradiation and EMS) of some biological parameters in M_1 generation and suggested that gamma irradiations were more effective than EMS and the cultivar KS-1 was more sensitive to mutagens under the tested doses and concentrations.

Datta and Rang[165] screened seven viable morphological mutants (*lax branching, feathery leaf, bushy I* - Fig. 82, *bushy II* - Fig. 83, *lax pinnae* - Fig. 84, *needle leaf* and *crumpled leaf*) from 7956 treated plants at M_2 following mutagen treatments (gamma-rays, EMS and H_2O_2 and their combined treatments) to dry seeds (moisture content: 7.5%). F_2 segregation (control × mutant, F_1 normal) revealed that *lax branching, feathery leaf, bushy I* (associated traits: synchronous flowering, compact habit, thick dark green pinnae of leaves), *bushy II* (thick dark green pinnae of leaves) and *lax pinnae* (pinnae elongated) mutant traits were controlled by a single pair of recessive genes; while, selfed lines of *needle leaf* and *crumpled leaf* mutants showed that the mutant traits were controlled by two pairs of recessive genes.

Datta and Rang[166] spotted a viable *chloroxantha* mutant in EMS treated population at M_2. The seedlings of *chloroxantha* (Fig. 86) were pale greenish yellow in color (2012 – "Dictionary of Colour" by Maerz and Paul 1950) and the mutant could be easily marked at the very seedling stage. The mutant plants showed delayed flowering (17 to 29 days from control plants) and maturity, which indicates that the mutant being deficient in chlorophyll content might have utilized their buffering capacity to maintain the photosynthetic efficiency by increasing the number of branches (consequently pinnae of the leaves increased in the mutant) and duration of the crop to complete their life cycle successfully. The inheritance of the mutant trait was recessive and was under the control of two gene loci. The mutant was compared with control at M_4 and results indicated that *chloroxantha* possessed higher number of primary branches and capsules per plant and had smaller seed (length) than normal; although, other traits were more or less comparable to normal plants. The authors presumed that the color of *chloroxantha* may be exploited as genetic marker for efficient breeding.

Rang and Datta[167] spotted five dark reddish brown (color code - 3/2), one yellowish brown (5/4) and one peach (512/1) color (colors were confirmed from Horticultural Color Chart 1968 and Munsell Soil Color Chart 1975) seeded plants at M_2 following different treatments of gamma irradiations and EMS. Mutation frequency of dark reddish brown color (Fig. 89), yellowish brown color (Fig. 88) and bicolor (peach color was associated with blackish tinge at the base and the apical region – therefore designated as 'bicolor' - Fig. 90) was estimated to be 1.92, 0.055 and 0.54 percent respectively (7956 plants scored). Dark reddish brown and yellowish brown seed-coat color traits were monogenic recessive to black seeds (Figs. 87-90); while, the inheritance of bicolor trait of seeds was under the control of two pairs of recessive genes (mutant × normal – reciprocal crosses were performed, F_1 – black and F_2 segregation analyzed following χ^2 – test analysis). Crossing experiments suggested that black coloration of seeds is dominant over other seed colors and gene symbols assigned were B for black, b^{dr} for dark reddish brown and B^y for yellowish brown colors and p for peach color of seeds, and the dominant form (P) of this gene has no effect on B or on any allelic forms of B (b^{dr}/b^y) and the mutation involving both the dominant genes (B-P) results to bicolor seeds. Following genotypes were proposed for the seed-coat colors – BBPP, b^{dr} b^{dr} PP, $b^y b^y$PP and bbpp for black, dark reddish brown, yellowish brown and bicolor seeds respectively. The true breeding mutant plants were evaluated at M_4 in comparison to control for several agronomic traits and it was noted that dark reddish brown seed coat

mutant was as productive as normal; while the bicolor and the yellowish brown seed coat mutants were sort sized and small seeded plants.

Polygenic Mutation: Datta and Biswas[168] analyzed variations for quantitative characteristics (plant height, number of primary and total branches per plant, total capsules, capsule chamber/fruit, capsule length and seed per capsule) from 10 randomly selected plants of each of the M_2 (X-irradiated and EMS treated) lines and computed mean and coefficient of variations and also determined student t-test between control and treatment. The magnitude of variability released (as evidenced from C.V.) through induction of mutation was both positive as well as in negative direction, thereby suggesting random nature of mutation.

Biochemical Studies on Induced Mutants: Electrophoretic characterization and evaluation of seed protein in control and EMS induced mutant line of the species were performed from seed samples[169] and the qualitative as well as quantitative variations in banding pattern among the plants were noted. The authors were of opinion that electrophoretic characterization of the mutant lines may be used as an additional parameter to supplement cytogenetic data in understanding genetic variations. Das *et al.*[170] extracted protease from germinating seeds of wild type and seven EMS induced mutant lines of *N. sativa* and the activity was assayed with casein as substrate in the pH range 3.5-8.0. Results indicated that most protease types showed pH 3.6-7.0 and more than one protease enzyme in the plant types tested. Amylase activity and variation of amylase isozyme pattern were also studied and it was reported that gene(s) controlling enzyme production/activity have been affected differentially in different mutants.

Cytogenetical consequences of induced mutagenesis

Translocation Heterozygosity: Datta and Biswas[171] isolated a cytologically marked plant (phenotypically indistinguishable) from the R_1 population of gamma irradiations, which showed ring or a chain quadrivalent in 49.38% meiocytes at MI (241 cells scored). Although normal 6II formation was noted predominantly (50.72%) at MI, the most common type of configuration studied in the marked plant was 4II+1IV (34.25%); while in the remaining meiocytes the quadrivalent appeared in association with 2 univalents. PMCs with ring of four chromosomes (41.08%) occurred more frequently than those with chain quadrivalent (8.3%). Among the meiocytes showing interchanged configurations, 65.55% were alternate and 34.45% were with adjacent orientations. Anaphase I separation was mostly (82.0%) balanced (6/6) although pollen sterility was high (55.8%) with extremely poor seed setting (12.2±5.7) per capsule as compared to normal (pollen sterility – 2.2 to 3.6%; seed setting 65.6±4.2/capsule) plants.

Datta and Biswas[172] screened four extremely dwarf plants at M_3 having identical leaf phenotype as their progenitor from the selfed M_2 *feathery leaf* mutant (0.50%, 2 hour EMS treatment). Meiotic studies revealed the characteristic presence of paired fragments in the parent (M_2) and multivalents in the *dwarf* mutant plants (M_3 as well as M_4). The dwarf plants were designated as *telescopic* mutants as the leaves were found to be clustered around the stem forming a crown-like appearance. Out of four *telescopic* mutant (Fig. 91), one of which showed prevalence of ring quadrivalent. The cytogenetically marked *telescopic* mutant was semisterile and the possible origin of the mutant lines has been ascribed due to deficiency of genes as an outcome of chromosomal deletion in the parent.

Saha and Datta[145] induced 5 translocation heterozygotes (P-14 and P-26 from 5 kR and P-32, P-36 from 10 kR) following gamma irradiations (5, 10 and 20 kR) to dry seeds (moisture content 7.5%). P-14 (possessing long drooping floral shoot), P-32 (lax branching) and P-36 (semi-dwarf with thick and non-shattering capsules) were viable translocations; while, P-26 and P-37 yielded only abortive seeds at R_1 following selfing and on open or controlled pollination. The translocation heterozygotes exhibited the formation of either a ring or a chain of 4 chromosomes in 38.7% to 77.7% meiocytes apart from 6II formation (Figs. 92-101). Predominance of rings occurred in all translocation heterozygotes excepting P-26 where rings and chains were nearly equal. P-14 and P-26 had more adjacent orientation of quadrivalents than alternate; while, P-32, P-36 and P-37 demonstrated random orientations. The quadrivalent behavior was found to be persistent in all generations (R_1, R_2 and R_3) of P-14, P-32 and P-36. The rings showed preponderance of adjacent orientation and the chains demonstrated frequent alternate orientation. Though normal 6/6 separation of chromosomes at AI was observed in 85.8, 83.3, 69.4, 82.3 and 86.4% cells of P-14, P-26, P-32, P-36 and P-37 respectively (rest showed unequal separation of chromosomes and bridge formation with a lagging fragment - Figs. 102-103), pollen fertility was reduced in the heterozygotes (8.2 to 37.5%). F_1's raised from intercrossing of P-14, P-32 and P-36 were meiotically assessed and the results indicated that same 2 non-homologus chromosomes were involved in translocation and the 2 longest pairs were suggested to be associated.

Desynapsis (Synaptic Mutants): Datta and Biswas[173] noted desynaptic behavior of chromosomes in a *bushy* mutant (M_2 generation, 0.5% EMS, 2 hour treatment) and the mutant trait was reported to be controlled by a single pair of recessive genes. The *bushy* mutant plant could always be characterized by their delayed germination, flowering and maturity, high frequency of sterile pollen grain formation, poor seed setting and univalent formation in the meiocytes. Desynapsis

studied in the mutant was partial or weak because of high frequency of bivalents per cell (4.70 to 5.24) than univalent per cell (1.53 to 2.59). Compared to controls (5.24 ± 0.41 chiasma/cell), frequency of chiasma has been found to be decreased in M_2 *bushy* mutant (4.57 ± 0.49 /cell). Univalents formed in the mutant line were found to be distributed randomly in most of the cases, which were not affected by the number of bivalents per cell. Less frequently, however, occurrence of univalent in close proximity to each other could be marked, which may be an indication of their belonging to same pair and their very recent separation. Anaphase I separation was irregular (34.44% to 44.23%) in the mutant line leading to the formation of laggards and unequal separation of chromosomes. Lagging chromosomes at anaphase II and unequal size of microspores in tri- and polysporous condition were also noted.

Saha and Datta[174] reported two synaptic mutants (DS-1, 5 kR gamma irradiations; DS-2, 10 kR) at M_2 (screened from 6582 M_1 plant progenies) possessing distinctive phenotypic marker trait (lax branching). The synaptic mutants (medium strong type) demonstrated fuzzy appearance of chromosomes at early prophase I (Figs. 104-105) along with univalent frequency ranging from 0 to 12 (enhanced frequency - Figs. 106-112) per cell (control: 0.10, DS-1: 2.47, DS-2: 3.50), reduced number of chiasma and bivalent per nucleus (control: 5.95 II/cell, chiasma 9.34 ± 0.3; DS-1: 4.77 II/cell, chiasma 7.40 ± 0.3; DS-2: 4.25 II/cell, chiasma 5.59 ± 0.4), few meiocytes with unequal separation (5/7, 5-1-6 and 4/8) at AI (control: 0.5%, DS-1: 15.6%, DS-2: 22.5%), cytologically balanced AII cells and high pollen fertility (control: 98.06%; DS-1: 96.57%; DS-2: 93.95%).

Male Sterility: Male sterile mutants with distinctive phenotypic marker traits (*bushy*- EMS treatment; Datta and Biswas[175]; chlorophyll deficiency- 6 hours 0.25% EMS - Fig. 113; Rang and Datta[176]; *dwarf*-chlorophyll deficiency - Fig. 116, *crumpled pinnae* - Fig. 115, *bushy* - Fig. 114 and *lax pinnae*, gamma irradiation- 5, 10 and 20 kR and EMS- 0.25, 0.50 and 1.00%, 3 hours; Datta and Saha[177]) were isolated from M_2 mutagenized population. Concomitant association of phenotypic marker trait(s) with male sterility was unique as it not only give selective advantage but will also be of immense value in the breeding behavior of the crop.

Datta and Biswas[175] isolated a male sterile mutant which was indistinguishable at earlier stages of growth, but the mature plant could be recognized by its characteristics dark green, thick and leather like pinnae of the leaves and synchronous flowering. Although the mutant demonstrated normal behavior of meiotic chromosome with 6 bivalents in MI cells and usual formation of tetrads, none of the pollen grains could be scored in the mutant which is an indication of complete inhibition of pollen grain development leading to male sterility. Post tetrad developmental disturbances might be responsible for arrestation of pollen formation.

Rang and Datta[176] reported a male sterile plant which exhibit broad elongated lax pinnae along with yellowish green pinnae in the shoot apex of the primary axis at the onset of floral bud initiation (Fig. 113), and non-dehiscent and pollenless anthers at anthesis. The male sterile plant showed desynaptic behavior of chromosomes and the chromosomal association studied at diplotene, diakinesis and MI (168 PMCs scored) were 6II (4.76%) - Fig. 117, 5II+2I (9.52%), 4II+4I (4.76%) and 12I (80.95%) - Fig. 118-119. Mean frequency of univalents and bivalents per cell was estimated to be 10.10 and 0.90 respectively, chiasma frequency per nucleus observed in the male sterile plant was 0.31±1.2 as compared to 9.9±0.74 in normal plants. Unequal separation of chromosomes was studied in AI (71.43%) and AII (42.62%) from 82 and 122 cells respectively. The male sterile plant produced tetrads mostly with unequal spory (Fig. 120) followed by near complete degeneration of microspores (Fig. 121) compared to normal oval shaped fertile pollen grains in control plants (Fig. 122).

Datta and Saha[177] categorized male sterile mutant plants into five (I to V) types on the basis of sterility and morphology. The mutants were type I: mutant *dwarf*, pollen grains 100.0% sterile and showed sign of degeneration, pollen grains were round and small sized 27.2 µm × 24.8 µm (Figs. 128-129) as compared to oval shaped pollen grains (Fig. 130), 39.0 µm × 38.08 µm; type II: mutant yellowish green color, 100.0% sterile pollen grains, small roundish with thick wall; type III: mutant with crumpled and deformed pinnae of leaves, anther small sized 3.74 mm ± 0.05, brownish, shrunken and indehiscent and were completely pollenless at maturity. Meiotic analysis revealed no clear bivalent formation, rather the chromatin agglutinated into unequal masses (Figs. 123-125). Agglutination of microspores was also evident which consequently degenerated (Figs. 126-127); type IV: *bushy*, normal cytological behavior, 100.0% sterile pollen grains; type V: the mutant plants were with long elongated and dissected pinnae of leaves and the pinnae were lax in nature. The mutant plants demonstrated normal meiotic chromosomal behavior and formed tetrads but the pollen grains were completely sterile. The male sterile mutants showed monogenic recessive (IV to V) as well as digenic recessive (II) mode of inheritance pattern. Type I and III were both male and female sterile. The mutants arising out of gene mutation and the mutant genes have favoured the continuation of meiosis and thereafter they have acted on microspores and on pollen grains. The mutants were non-structural nuclear type as per classification proposed by Gottschalk and Kaul[178] and Johns *et al.*[179].

Trisomic: Datta and Biswas[180] isolated a trisomic (detected after male meiotic studies) plant from the

selfed progenies of M_2 *lax branching* mutant at M_3. Morphologically the trisomic plant was weak with slender stem and drooping lamina at the seedling stage. At maturity the plant attained a height of 19.7 cm. Flowering in the trisomic was delayed by 10-11 days as compared to normal plants. Only four flower buds of the trisomic bloomed, while the rest dried up. Flowers were smaller in size and at maturity the stamens turned brownish in contrast to yellowish green color in the control and ultimately rudimentary capsules with abortive seeds were formed. The trisomic showed 6II+1 (87.8%) and 5II+ 1III (12.2%) chromosomal associations in 72 and 10 PMCs respectively (Figs. 131-133). At AI, either the extra chromosome appeared as laggard or has been incorporated in any of the two poles (Fig. 134). The aneuploid plant appeared to be a primary trisomic, with 58.17% pollen sterility and it was completely seed sterile.

Cytomixis: Datta and Biswas[180] studied transfer of nuclear materials from one PMC to another at prophase I and MI while performing male meiotic analysis in M_2 mutants (observed in *lax branching* mutant). Chromatin transfer between adjacent meiocytes occurred through cytoplasmic links and the migration was at random within a group of PMCs (Figs. 135-137). The phenomenon of cytomixis was restricted between/among few clusters of meiocytes of a single microsporophyll squash preparation. Cytomixis resulted in hypo- and hyperploid variation in chromosome numbers (19.87%) in meiocytes, thereby producing aneuploid and polyploid PMCs. The nucleolus of the meiocytes, undergoing chromatin transfer, in most cases remained in the donor cell; rarely it passed to the cytoplasm of the recipient cell along with the chromatin materials. Clumping and sticky nature of the nuclear materials were also noted in certain PMCs.

Meiotic Instability: Datta and Biswas[181] identified a phenotypically aberrant and sterile plant at M_3 in the selfed progeny of EMS-induced M_2 mutant (*lax branching*), which showed aneuploid variation in chromosome numbers. Phenotypically, the aberrant plant exhibited lax branching nature (Fig. 138) attaining a relatively shorter height (32.7 cm) at maturity as compared to rather erect (43.65 cm ± 1.72) and compact habit of the normal plants. During the initial growth period of the plant the pinnae of lamina were represented by linear, thicker appendage like structures and at the latter stages few normal leaves developed. Most of the flower buds terminated in rudimentary flowers excepting a few which bloomed after 121 – 137 days after sowing instead of 70 – 98 days in control plants. The flowers had only 1–2 normal looking stamens, while rest of the microsporophylls were represented as leafy projections. These flowers produced only rudimentary capsules with abortive seeds. Meiotic analysis revealed distinct chromosomal instability - Figs. 139-143 (2II+5I – 2.6%, 5II – 5.3%, 11I – 2.6%, 5II+1I – 7.9%, 4II+3I – 7.9%, 6II – 26.3%, 1IV+ 4II+2I – 5.3%, 7II – 5.3%, 12 II – 36.8%, 78 PMCs could only be analyzed) with remarkably higher pollen sterility (78.5%). Both stained and unstained pollen grains were considerably smaller sized (10.05 µm ± 0.2; normal – 39.8 µm ± 0.6) than control pollen grains (Figs. 144-145). Moreover, functional instability of the stained (fertile) pollen has been evidenced by the formation of only rudimentary seeds in the marker plant. The aberrant has been ascribed as the outcome of cytomixis noted in *lax branching* M_2 mutant.

Induced polyploidy

Biswas and Chatterjee[182] induced tetraploid plants following seed and seedling treatments with various concentrations of colchicine and the plants were with increased number of branches, enhanced size and frequency of stomata, increase in the number of flowers, variation in pollen size, fruit setting and the rate of germination of seeds, increase in the number of septa per fruit and seeds per septum and delayed flowering. Biswas and Datta[183] performed meiotic analysis in colchicine induced (seedling treatments) autotetraploid plants and found prevalence of chromosome irregularities producing varying number of quadrivalents (0-4), trivalents (0-2) and univalent (0-10). The tetraploids were seed sterile.

Saha and Datta[184] induced one autotetraploid (C_0-1; 5 hour treatment with 0.5% aqueous solution of colchicine for 3 consecutive days) following treatment with colchicine at the apical meristematic tips of young seedlings bearing only two cotyledonary leaves. The autotetraploid at maturity yielded 37 healthy seeds, 30 seeds were sown in C_1 generation and 11 plants were obtained of which 4 were cytologically confirmed to be autotetraploids. The seeds of C_1 tetraploids were bulked and 25 randomly selected healthy seeds were sown in C_2 generation from which 5 plants were obtained and all were meiotically confirmed to be tetraploids. The most prominent morphological changes of C_0-1 tetraploid and its progenies at C_1 and C_2 were increase in flower and capsule sterility and reduction in seed number per capsule and seed fertility (expressed as per cent of control). Seed set in the tetraploid plants varied from 0.64 to 12.62% of control, thereby demonstrating negative selection value of induced autotetraploids. However, one autotetraploid (C_2-2) possessed some useful traits compared to the diploid and other tetraploids (Figs. 146-147). The C_2-2 plant yielded 118 good seeds (12.62% of control) and the flowers (significantly larger than those of diploids) of the plant (synchronous flowering) remained in blooming stage for a considerably long period (25 to 32 days) than the flowers of diploids (4 to 5 days) and the other tetraploids (8 to 12 days) studied over two generations. Compared to normal (Fig. 148) diploids ($2n$=12) the induced tetraploids ($2n$=$4x$=24) formed

quadrivalents (0-4), bivalents (1-12) and univalent (0-14) in varying proportion at MI (Figs. 149-151). Trivalents (0-2) were only observed in C_0-1 plant. The induced tetraploids formed 0.80 to 2.08 quadrivalents per cell and the coefficient of quadrivalent realization was low. Chi-square test of heterogeneity revealed that the frequency of bivalents and quadrivalents per cell among the tetraploids was random ($p>0.05$) but number of univalent per cell was non-random ($p<0.001$). The mean chromosomal association in C_2-2 was 1.37IV+9.00II+0.50I (32 PMCs scored). The univalent frequency among tetraploids demonstrated significant positive correlation with abnormal AI (laggards, bridges, groupings and unequal separation - Figs. 152-158) cells ($r=0.81$; $p<0.05$). Anaphase II cells showed unequal and multisporic conditions (Figs. 159-160).

The abnormal AI cells showed significant negative correlation with pollen fertility ($r=-0.99$, $p<0.001$). However, the correlation between frequency of abnormal AI cells and seed set and between pollen fertility and seed yield and between pollen fertility and seed fertility were non-significant. Cytological examination of induced autotetraploids leads to the conclusion that reduction in pollen fertility was the result of chromosomal disturbances arising from pairing irregularities. Seed sterility seems to have a genetical rather than cytological basis.

Genetic variability

Datta[147] studied relationship between yield and its attributes (plant height, number of primary branches/plant, total capsules/plant and seeds/capsules) and found significant positive correlation in all cases excepting for seed/capsule. Plant height was positively associated with primary branches/plant ($r=0.71$, $p<0.01$) and total capsules/plant ($r=0.69$, $p<0.01$); while, number of primary branches/plant was significantly associated with capsules/plant ($r=0.80$, $p<0.01$). However, capsules/plant showed insignificant relationship with seeds/capsule ($r=0.04$, $p>0.05$). Path coefficient analysis revealed that the direct contribution of total number of capsules/plant ($P_{35}=0.7460$) was very high and the trait indirectly contributed in high amount through plant height and number of primary branches. Direct contribution of plant height ($P_{15}=0.2886$) and seeds/capsule ($P_{45}=0.1296$) to yield was relatively low. Primary branches/plant ($P_{25}=-0.3374$) showed negative contribution to yield. Results indicated that capsules/plant is the most important trait for selection and crop improvement.

Iqbal *et al.*[185] studied 34 accessions with 2 check genotypes of black cumin for assessment of mineral nutrients. High variation was recorded for Fe, Ca, Cu, Mg, Pb, Zn, Co, Mn, Na, P, B, K and N amongst genotypes suggesting sample selection based on the composition of mineral nutrients. Correlation studies revealed significant association between Cu and Ca, and between Mg and Ca and Mg and Cu. Based on principle component analysis (PCA) six clusters were observed and it was suggested that the genotypes may be utilized in various combinations for genetic improvement of the species. Iqbal *et al.*[186] recorded genetic variation for plant height, days to first flower, days to 50% flowers, days to maturity, biomass, capsule weight, yield, seed weight and harvest index while studing 31 genotypes under field conditions with 3 replications. Three accession (MP00023, MP00111 and MP00120) were found better for more than one character and are expected to be a potential for improvement of *N. sativa*.

Tissue culture

Callus Induction: Banerjee and Gupta[187] raised calluses from leaf tissues and were of opinion that induction of calluses depended on the balance between auxin and kinetin in the medium and coconut milk factor as a source of kinetin. Chand and Roy[188] used different concentrations of 2,4-D, NAA and IAA to explore maximum callusing in the species. The concentration of kinetin has been kept constant throughout the experiments. The calluses grown in medium containing NAA, have been found to be friable, soft and green in color than in media containing IAA and 2,4-D. It was suggested that NAA was most favorable for producing callus tissue. Ghosh and Gadgil[189] initiated callus culture from excised hypocotyl segment when cultured in MS agar medium supplemented with IAA, NAA, IBA and 2,4-D. Chand and Roy[190] observed that in presence of GA in the media the seeds as explant grew into plantlet and there was no callus formation; while in the presence of NAA in the media seeds first produced calli from which plantlet developed. In the presence of IAA seeds grew into plants but at the base callus formation took place. In all cases amount of kinetin and coconut milk were kept constant. It was also pointed out that in presence of 2,4-D, kinetin and coconut milk seed proliferated into callus tissue without formation of plantlets. Datta *et al.*[191] reported calli formation from hypocotyl segment in MS medium supplemented with 2,4-D (2 mg/l) and kinetin (1 mg/l), and they were creamy white, compact ones. Youssef *et al.*[192] reported that 0.05 per cent casein hydrolysate promotes callus growth; however, growth was reduced by increasing salinity. On the contrary, it was also suggested that accumulation of primary products in callus cultures is enhanced by salt stress. Al-Ani[193] cultured roots, hypocotyls and leaves in MS medium supplemented with 2,4-D (0.0, 1.0, 2.0, 3.0, 4.0 mg/l) and kinetin (0.0, 1.0, 1.5, 2.0, 2.5, 3.0, 5.0 mg/l) and best callusing was obtained from leaf explants with 1 mg/l 2,4-D and 1.5 mg/l Kin. Such callus yielded higher thymol concentrations after 75 days by HPLC.

Suspension Culture: Banerjee and Gupta[194] reported that in suspension culture 91% free cells of *N. sativa* was obtained in WHITE's medium supplemented

with casein hydrolysate, inositol and adenine. Ploidy distribution pattern was similar in cell clumps of different sizes and free cells. Chromosomal irregularities were more in free cells. A number of globular embryoid were formed when casein hydrolysate, inositol and adenine were added in the medium after subsequent omission of auxin and coconut milk.

Embryogenesis: Banerjee and Gupta[195] noted embryogenesis in leaf callus (MS media supplemented with casein hydrolysate; coconut milk replaced). Casein hydrolysate suppressed the differentiating capacity at a concentration of 100mg/l after fifth subculture. It was reported that 2,4-D and kinetin have inhibiting effect on morphogenesis. On the histological examination of differentiated tissue, it was observed that roots, shoots, buds and leaves have originated from group of meristematic cells whereas embryoids have initiated by the repeated division of single cell.

Elhag *et al.*[196] with an objective of inducing and isolating somatic embryos for biosynthetic studies callus cultures were initiated from leaf, stem and root explants of axenic seedlings on MSB5 basal medium supplemented with kinetin (0.46 µM) and 2,4-D (4.5 or 13.5 µM) or NAA (5.4 or 16.2 µM) in the dark. Cultures initiated and subcultured on medium containing NAA produced friable callus with numerous roots regardless of explant type. These cultures differentiated into somatic embryos on medium containing NAA. The embryos developed into leafy structures on basal medium devoid of growth regulators. When the embryogenic callus was transferred to liquid medium containing NAA, numerous embryos and clusters of embryos were released into the liquid medium but, in contrast to solid medium, development remained arrested at the early embryonic stages.

Chromosomal Instability: Chand and Roy[188] reported very high number of chromosomes in media containing 2,4-D and kinetin; while NAA resulted very minor chromosomal variations. Ghosh and Gadgil[189] found shift in ploidy level from diploid to higher polyploids in presence of 2,4-D and when kinetin was mixed with 2,4-D or 2,4-D mixed with coconut milk factor. Bansal and Sen[197] reported that polyploidy has been a common feature of occurrence in calluses induced from root, shoot and leaf tissues and their appearance did not show marked difference in the tissues. Datta *et al.*[191] studied numerical variations in chromosome number including polyploidy, aneuploidy and haploidy as well as structural anomalies (Figs. 161-166) from callus tissues raised from hypocotyl segment. Frequent chromosome elimination in different cell lines was noted; however, the marker chromosomes (telocentric) were found constantly at different ploidy level. Kumar and Roy[198] were of opinion that apart from occurrence of high frequency of aneuploid and polyploid cells in callus tissues, structural anomalies like binucleate cell, micronuclei, diplochromosomes, multipolarity, sticky bridges and ring chromosomes formation was also observed. Anomalies might be due to endoduplication and various mitotic disturbances.

Molecular genetics

Al-Huqail and Al-Saad[199] performed DNA fingerprinting in 4 accessions from Saudi Arabia, Ethiopia, Egypt and Syria with an objective of genotypic characterization between/among black cumin taxa. Inter Simple Sequence Repeat (ISSR) method was employed in the PCR technique to detect genetic polymorphism. The scored bands of the DNA fingerprints (17 primers representing 3 types of intermicrosatelites – di, tri and tetra of short tandem repeats) were 108 in Saudi Arabia, 106 in Ethiopia, 100 in Egypt and 81 in Syria and the percentage of dissimilarity was computed to be 21.5-36.3%. Twenty four genes representing 24 different enzymes and isozymes were selected and scanned via PCR technique using suitable SSR primers and the obtained results showed some changes in the genetic structure of some of these genes. Iqbal *et al.*[200] carried out investigation to explore genotype specific fingerprinting of 32 germplasms based on randomly amplified polymorphic DNA markers. From 58 random primers used, 15 primers generated 249 reproducible and scorable amplification products across all the genotypes, out of which 164 (66.0%) fragments were polymorphic revealing a high level of polymorphism among the genotypes. The proportion of common bands was low (34.0%). In 13 genotypes, 27 bands of different masses (kilobases) were recorded and were considered specific. The specific/amplified PCR products were reported to be used as molecular markers for identification of germplasms and resource protection. The result of genetic polymorphism was validated from UPGMA and PCA.

Genes

1. APETALA 3 – like protein (AP3-3) mRNA, 746bp, linear, partial cds, accession – HQ694794[201].
2. APETALA 3 – like protein (AP3-2) mRNA, 865bp, linear, partial cds, accession – HQ694795[201].
3. PISTILLATA – like protein (PI-2) mRNA, 809bp, linear, partial cds, accession – HQ694796[201].
4. PISTILLATA – like protein (PI-1) mRNA, 840bp, linear, partial cds, accession – HQ694797[201].
5. microsatellite NIG_HSP 70 sequence, DNA, 345bp, linear, accession – HM803244.1[202].
6. *Nigella sativa* voucher A. Guener, M. Vural and H. Sagban 9189 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal

transcribed spacer 2, complete sequence, DNA, 621 bp, linear, EU699463[203].

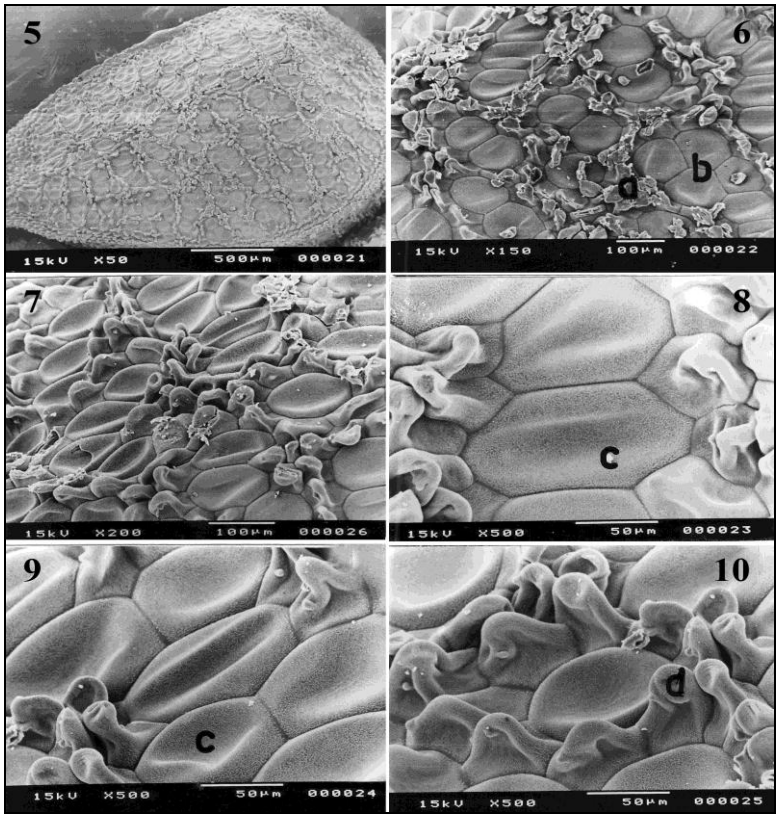
7. *Nigella sativa* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, DNA, 621 bp, linear, EU699464[203].
8. *Nigella sativa* beta-amyrin synthase (basl) mRNA, complete cds, mRNA, 2430 bp, linear, FJ013228[204].
9. *Nigella sativa* beta-amyrin synthase (basl) gene, complete cds, DNA, 4444 bp, FJ013229[204].
10. *Nigella sativa* squalene epoxidase 1 (seq 1) mRNA, complete cds, mRNA, 1566 bp, linear, FJ232947[205].

Patents

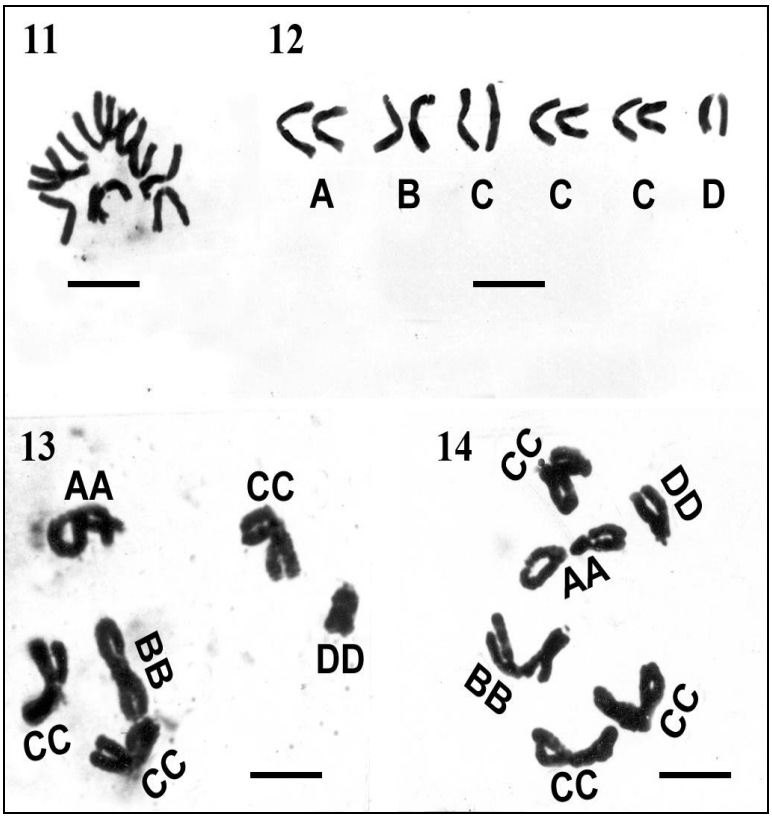
Nigella sativa currently has five FDA (Food and Drug Administration) separate patents in the U.S.A. for the treatment of:

1. Inhibition of cancer cell growth, Patent no.- US 5,653,981, Inventor- R. D. Medenica.
2. Diabetes, No.-US 6,042,834, Inventor – Wasif Baraka.
3. Improvement of the Immune System, No.- US 5,482,711, Inventor – R. D. Medenica.
4. Viral Infections, No.- US 6,841,174, Inventor – S. I. A. Shalaby and E. M. A. H. Allah.
5. Psoriasis, No.- US 6,531,164, Inventor – H. H. R. Credé.

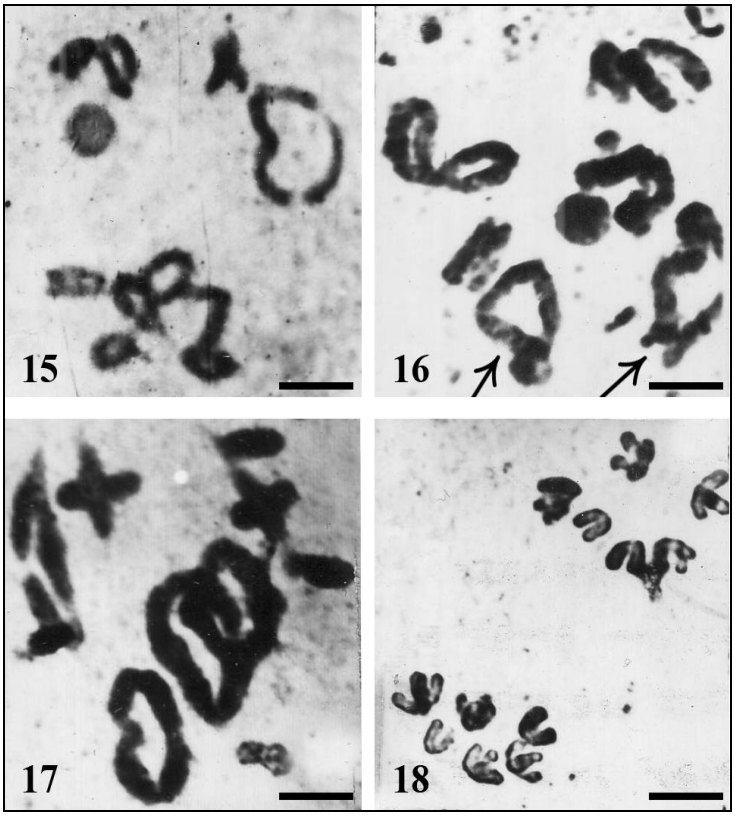
Figs. 1-4. 1) Normal *N. sativa* plant. 2) Flower before pollination. 3) Flower after pollination. 4) Seeds of black cumin.



Figs. 5-10. Scanning Electron Microscopy of seed surfaces of black cumin. [Source: Cytologia 68, 2003]



Figs. 11-14. Chromosomes (2*n*=12) in *Nigella sativa*. 11) Mitotic chromosome. 12) Photoplate ideogram showing 4 (AA, BB, CC, DD) chromosome types. 13-14) Diplotene plates where the bivalents are marked. Bar=15 µm. [Source: Cytologia 67(4), 2002]



Figs. 15-18. Meiotic configurations (2*n*=12). 15-16) 6II at diplotene. 17) MI showing 6II. 18)6-6 separation of chromosomes at AI. Bar=15 µm.

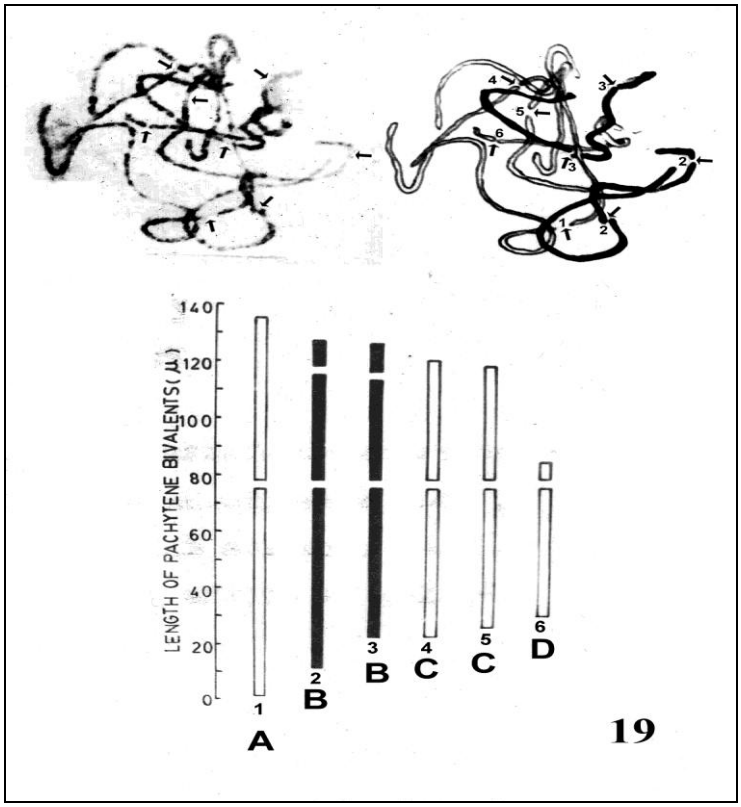
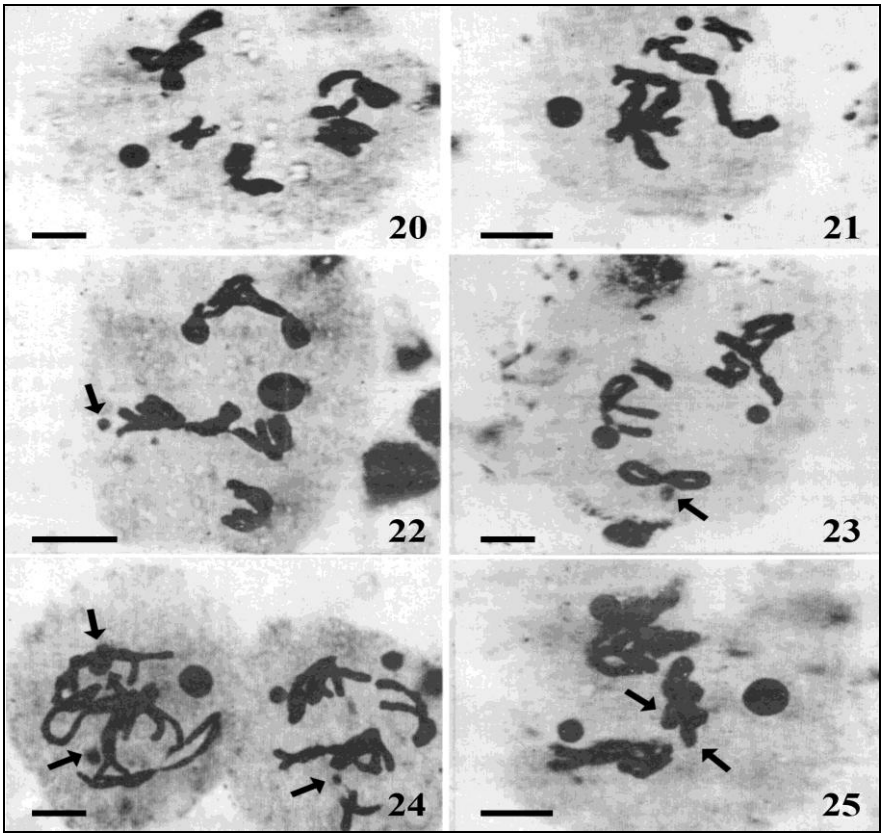
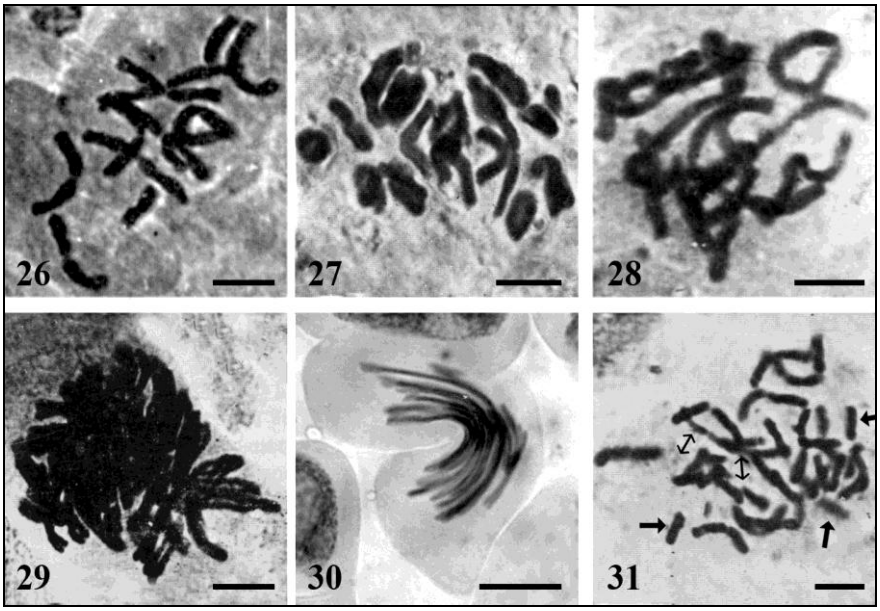


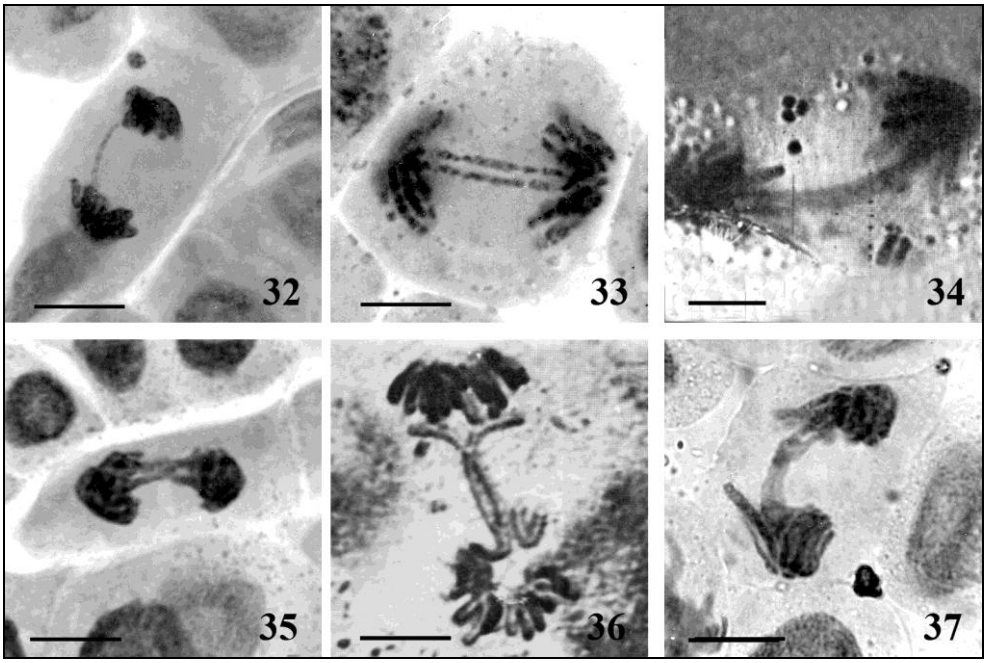
Fig. 19. Pachytene chromosome configurations.



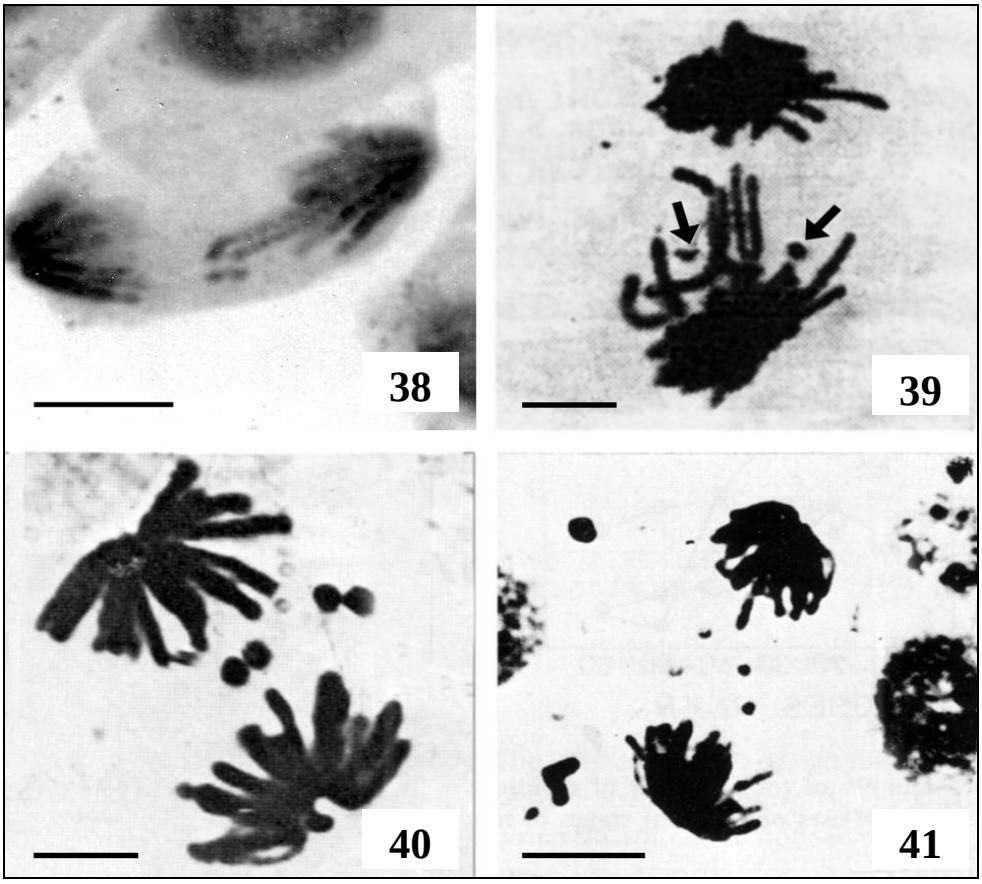
Figs. 20-25. PMCs at prophase I showing variation in number and size of nucleoli in control and in mutant lines of *N. sativa*. 20) One nucleolus. 21-22) Two unequal sized nucleoli, unattached to bivalents. 23) Three nucleoli. 24) Four nucleoli of which two are attached to a bivalent (a) and three unequal sized nucleoli (b). 25) Five nucleoli. Bar=15 µm. [Source: J. Phytol. Res. 12(1-2), 1999]



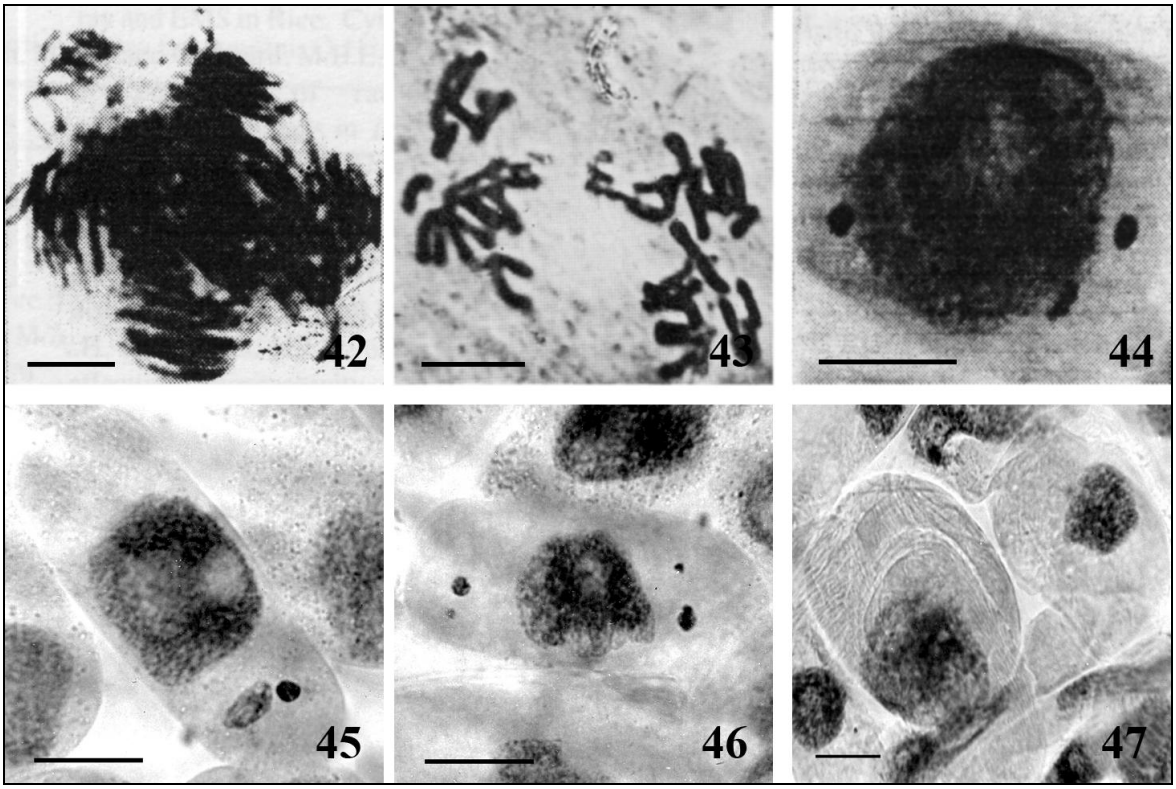
Figs. 26-31. Mitotic consequences following irradiations at metaphase. 26) 2n=12 – normal configuration. 27) Pseudochiasma like configuration. 28) Ring chromosome. 29) Diplochromatic nature of chromosomes in a polyploid cell. 30) Abnormal shaped cell with chromosome bending. 31) Aneuploid cell with fragments and unequal chromosome length. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



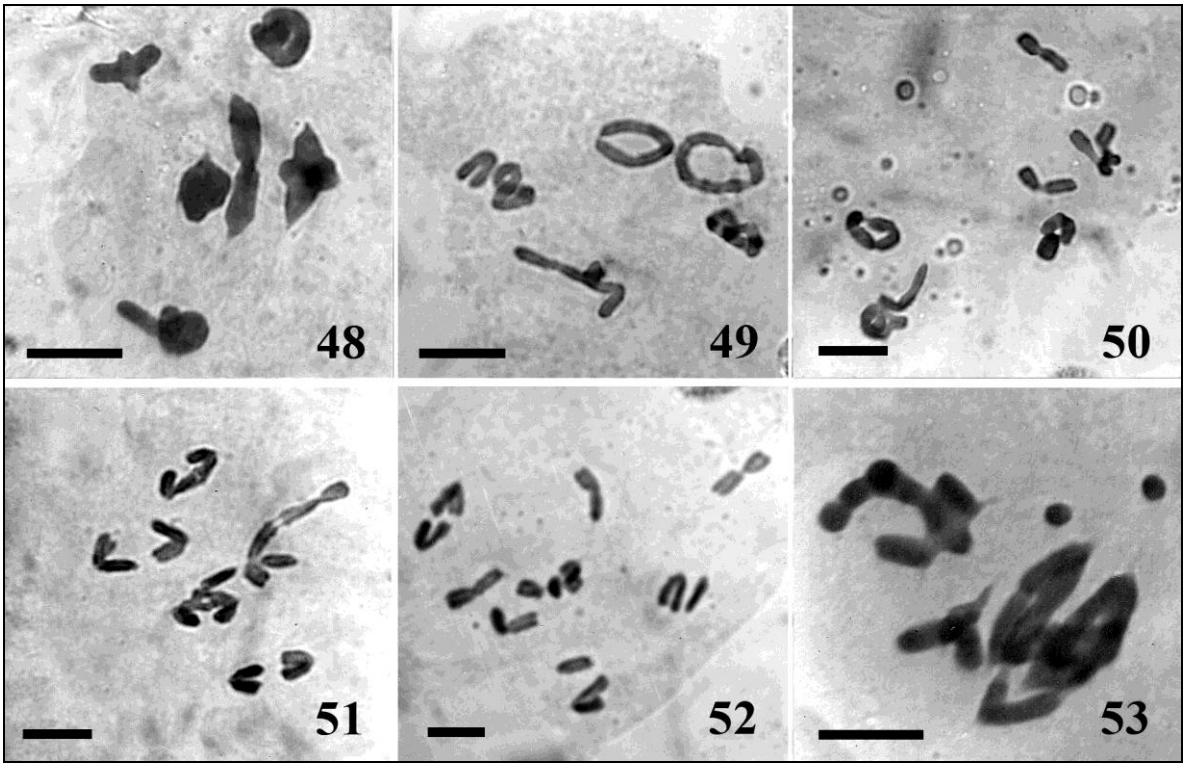
Figs. 32-37. Anaphase bridge formation in irradiated samples. 32) Single bridge with a round globular fragment. 33) Double bridge. 34) Double bridge with equal sized round and rod fragments. 35-36) Criss-cross bridge. 37) Interlocked bridge. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



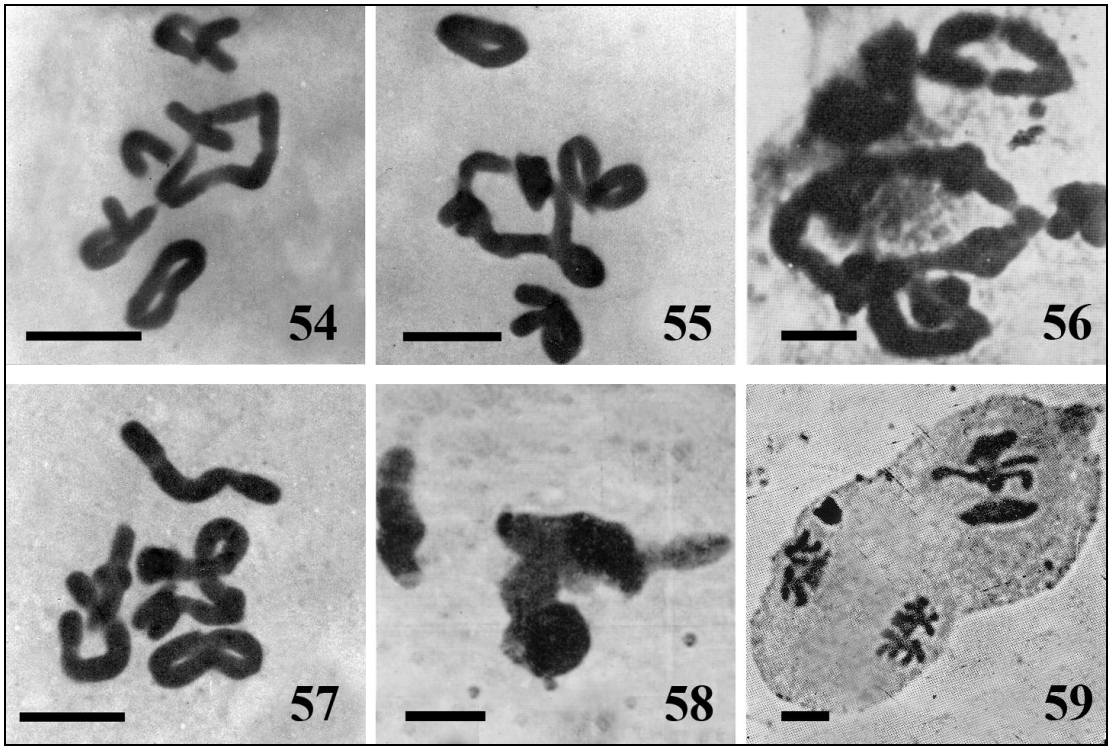
Figs. 38-41. Anaphasic events following irradiations. 38-39) Incomplete bridge with two identical sized fragments. 40) Paired fragments. 41) Four fragments. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



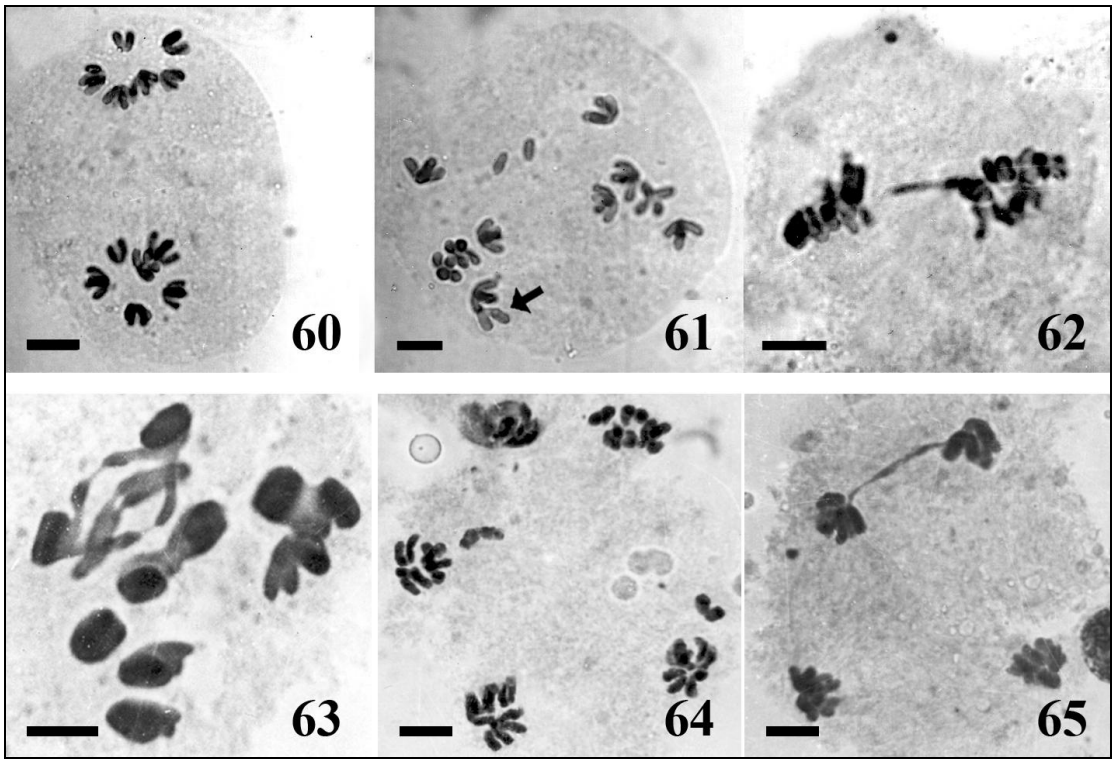
Figs. 42-47. Mitotic events following irradiations. 42) Polyploid cell at anaphase showing multipolar organization. 43) Multipolarity at anaphase. 44) Two condensed nearly identical sized micronuclei in resting cell. 45) Condensed and uncondensed micronuclei. 46) Four unequal sized micronuclei. 47) Giant cell. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Dev. Sci. 3(1), 2011]



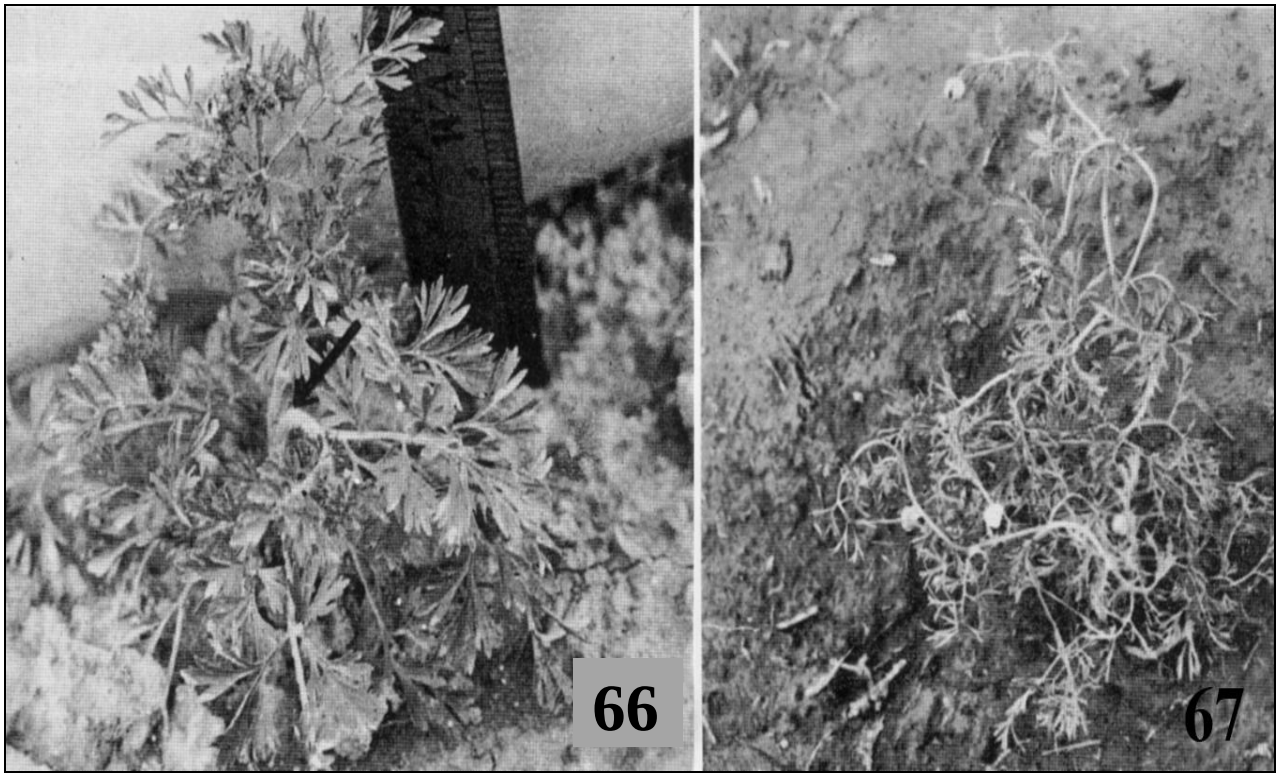
Figs. 48-53. Meiotic consequences of irradiations at metaphase I. 48-49) 6II. 50-51) 3II+6I. 52) 2II+8I. 53) 6II+2 identical sized fragments. Bar=15 µm. [Source: J. Plant Dev. Sci. 3(1), 2011]



Figs. 54-59. Meiotic events at MI. 54)1IV (adjacent orientation) + 4II. 55) 1IV (alternate) + 3II+2I. 56) 1IV (adjacent) + 4II. 57) 1IV (non-co oriented) + 4II. 58) Sticky configuration of chromosomes. 59) Fusion of two PMCs. Bar=15 µm. [Source: Cytologia 48, 1983; Cytologia 51, 1986]

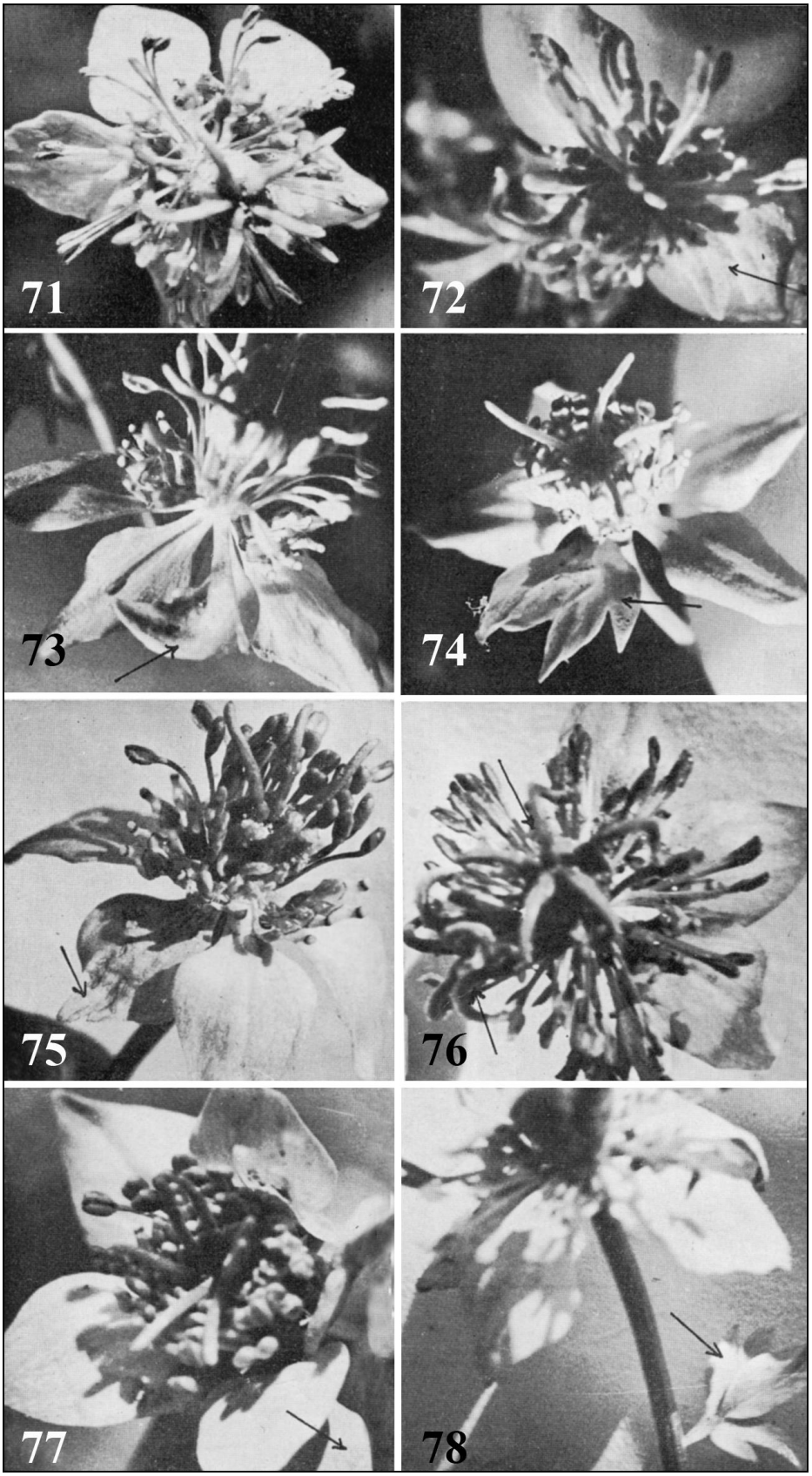


Figs. 60-65. Meiotic configurations in irradiated samples at AI and AII. 60) 6-6 separation at AI. 61) Two fragments at AI. 62) Dicentric chromatid bridge with an acentric fragment. 63) Double bridge formation at AI. 64)Two lagging chromosomes at AII. 65) A bridge with a fragment at AII. Bar=15 µm. [Figs. 26-65. Ref.: Cytologia 48, 1983; Cytologia 51, 1986; J. Plant Developmet Sci. 3(1-2), 2011]

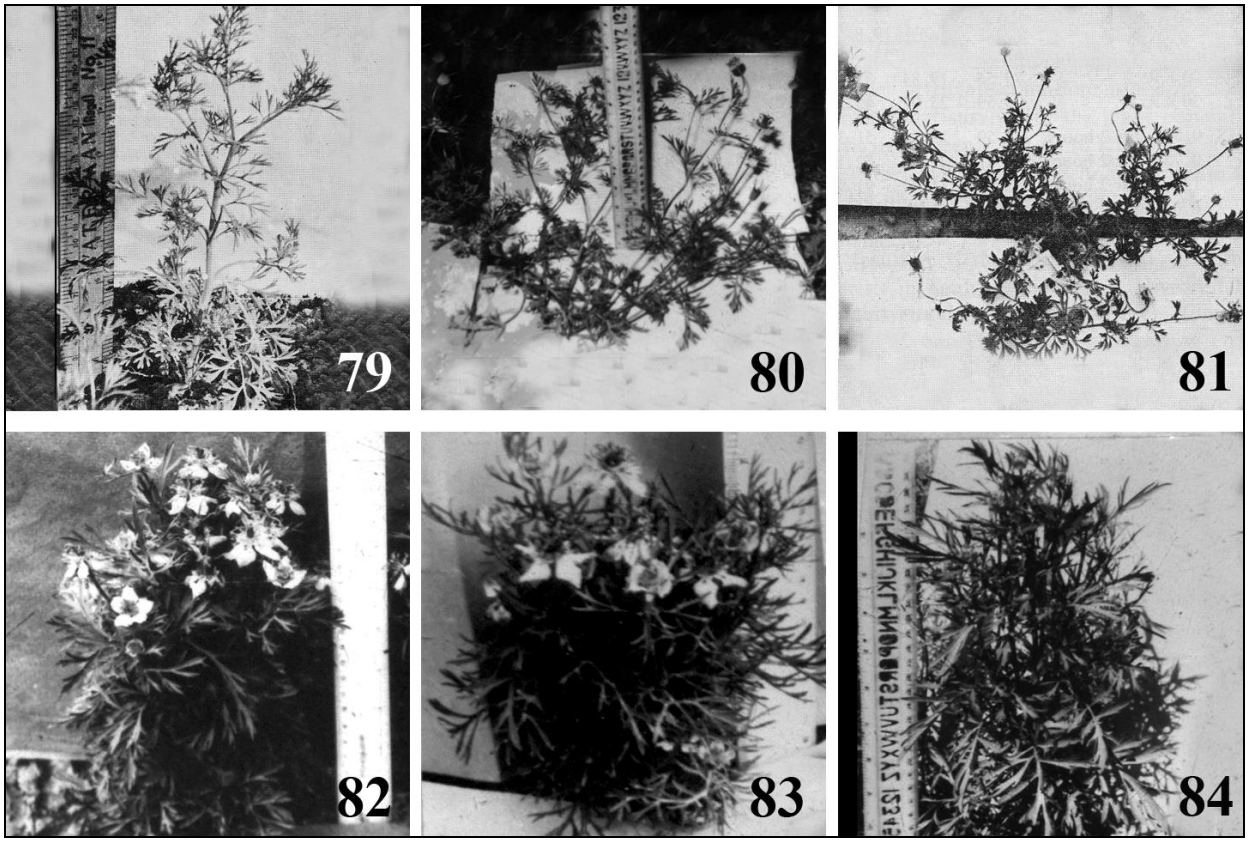


Figs. 66-67. Stem anomalies. 66) Bifurcation. 67) Twining nature. [Source: Cytologia 50, 1985]

Figs. 68-70. Stem abnormalities. 68) Trifurcation. 69) Twining. 70) Unbranched. [Source: Cytologia 50, 1985]



Figs. 71-78. 71) A normal flower. 72-78) Floral abnormalities. 72) Unequally dissected petaloid sepal. 73) Shield shaped sepal. 74) Triforked sepal. 75) Elongated and strap shaped petal. 76) Presence of two gynoecium in a same flower. 77) Small sized sepal in addition to the normal complement. 78) Incompletely forked bract like structure. [Source: Cytologia 50, 1985]



Figs. 79-84. Control and mutants of N. sativa. 79) Normal plant. 80) *Lax branching*. 81) *Prostrate*. 82) *Bushy I*. 83) *Bushy II*. 84) *Lax pinnae*. [Source: Cytologia 50, 1985]

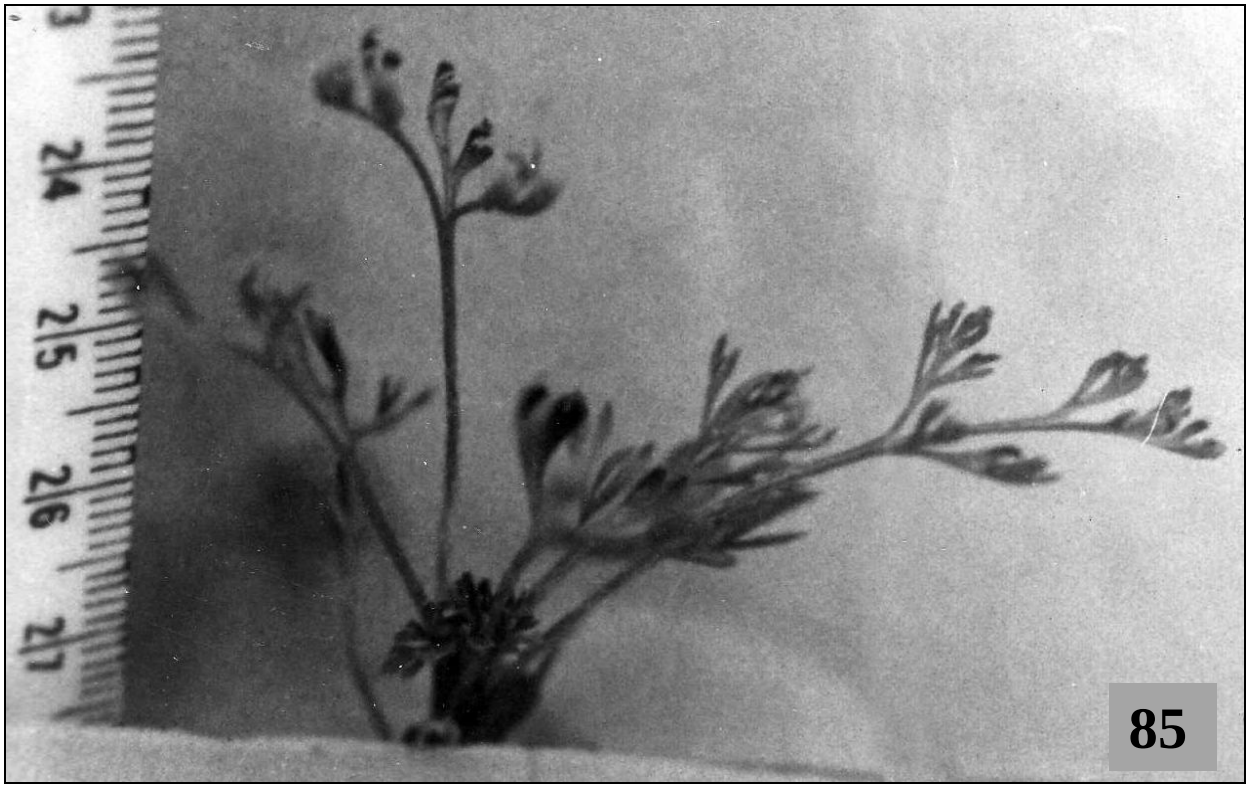
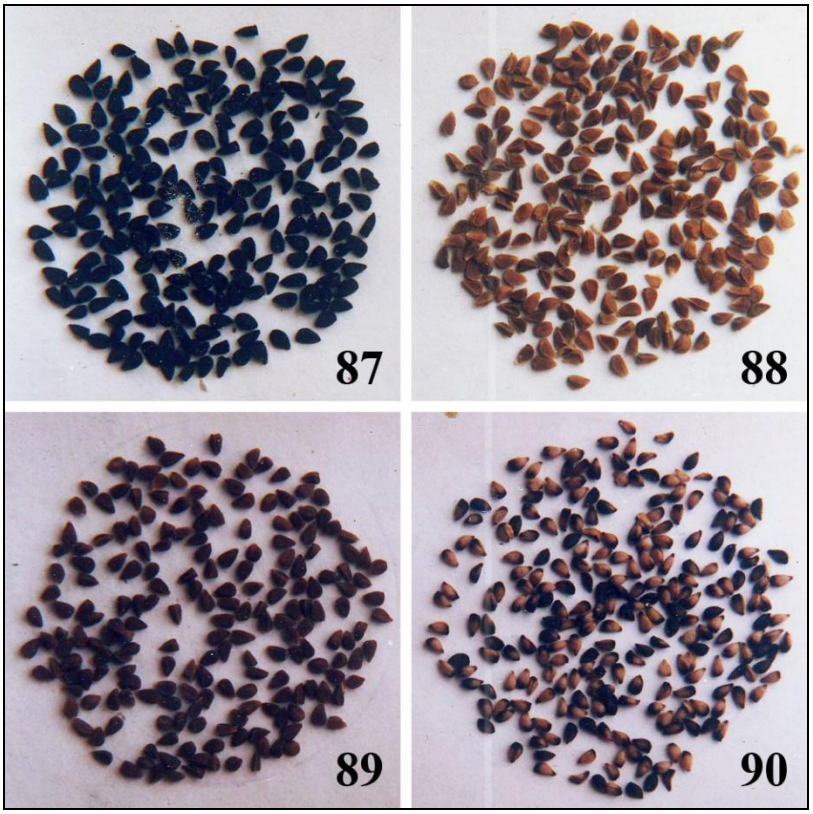


Fig. 85.*Cup leaf* mutant.

Fig. 86. *Chloroxantha* with normal plants in field condition. [Source: Ind. J. Genet. Pl. Breed. 61, 2001]



Figs. 87-90. Seed-coat color in *N. sativa*. 87) Black in normal. 88) *Yellowish brown*. 89) *Dark reddish brown*. 90) *Bicolor*.

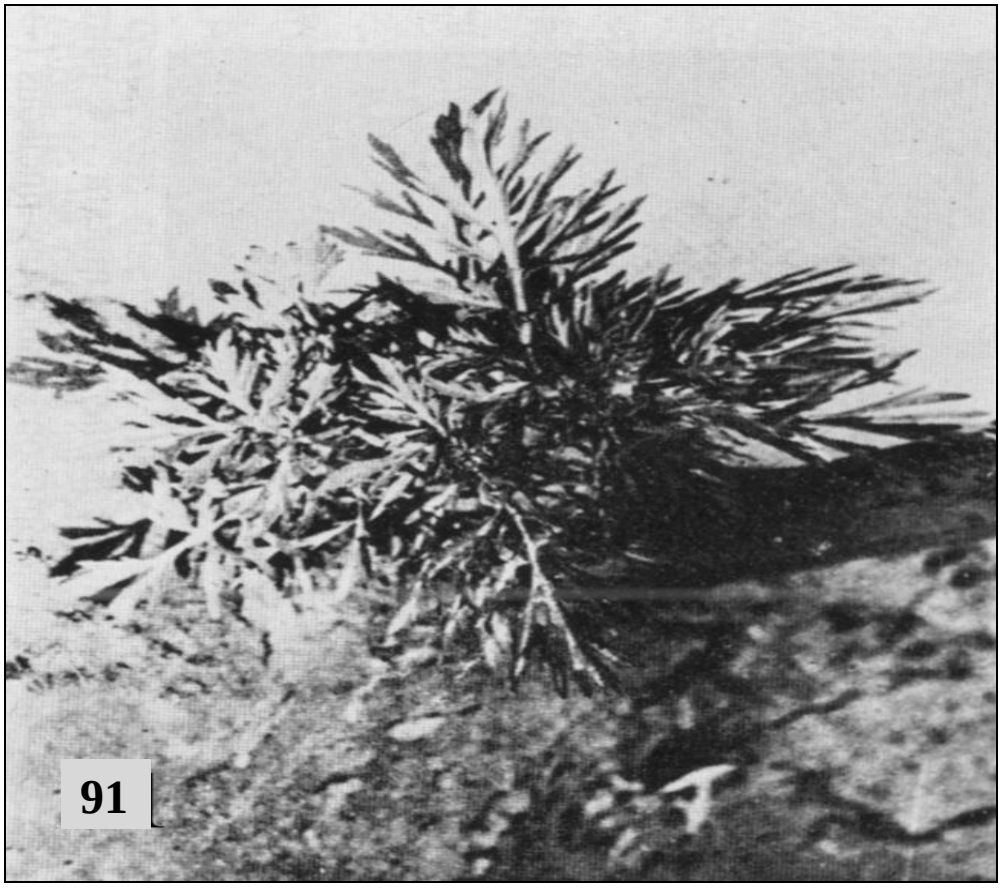
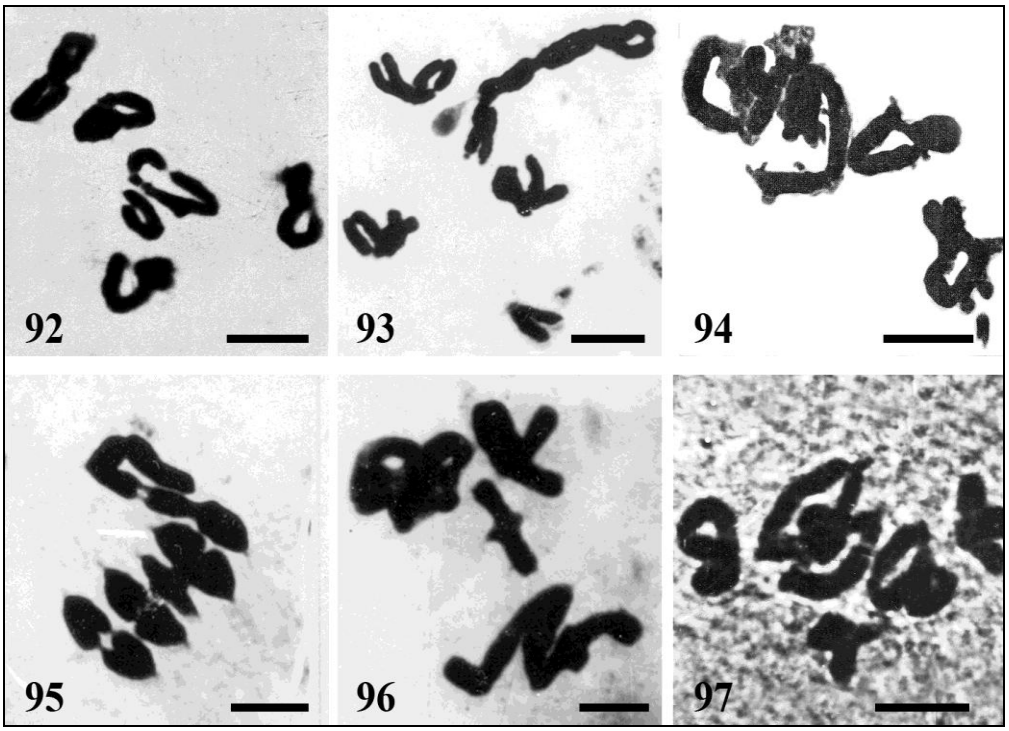
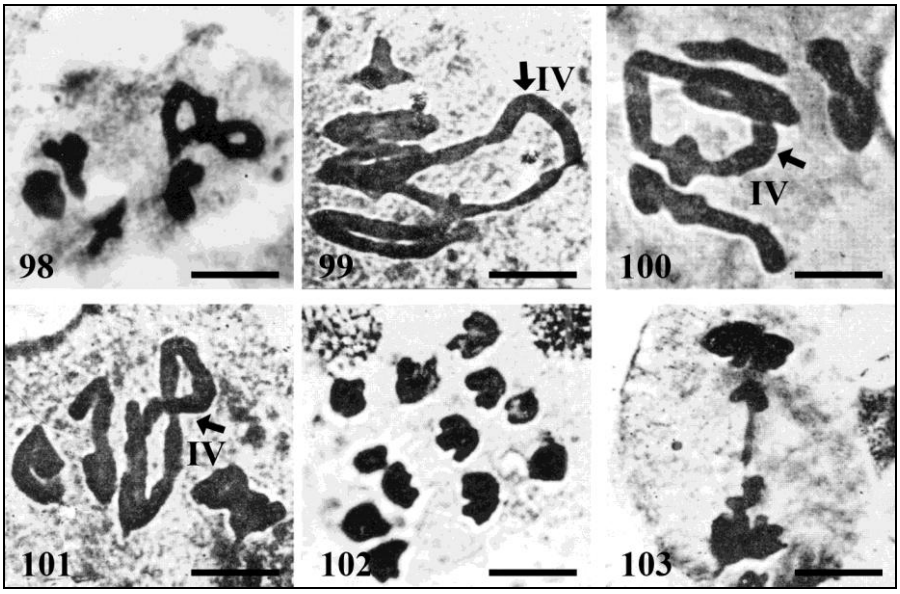


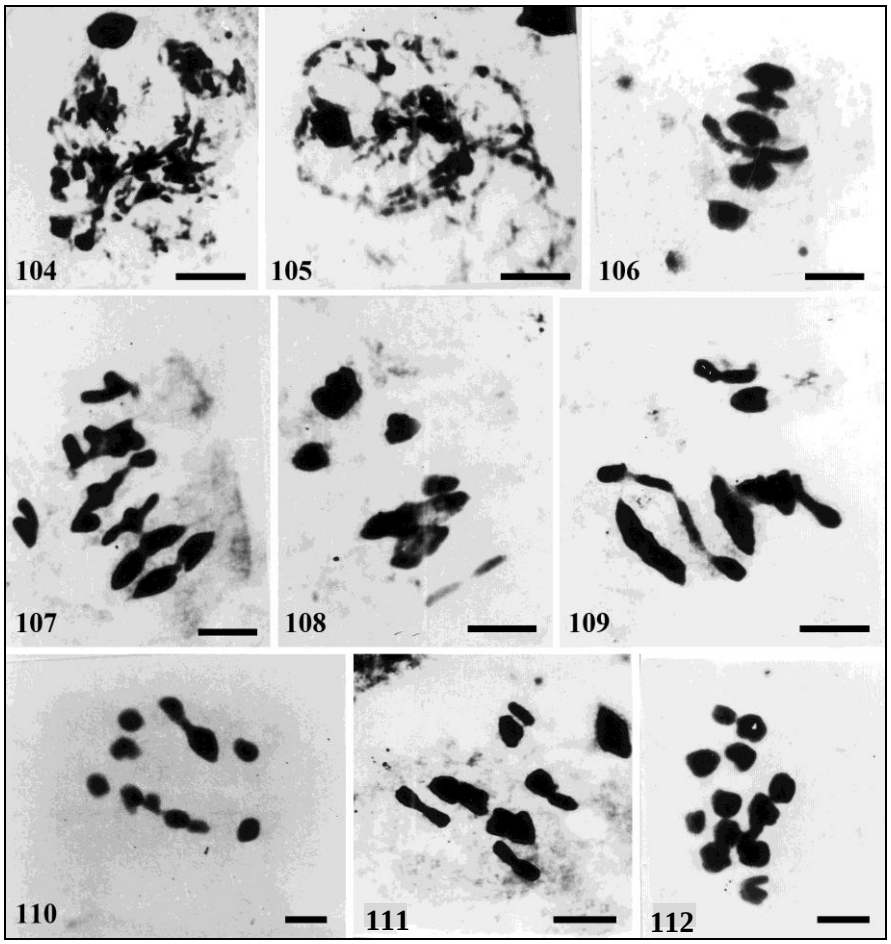
Fig. 91. *Telescopic* mutant in *N. sativa*. [Source: Cytologia 51, 1986]



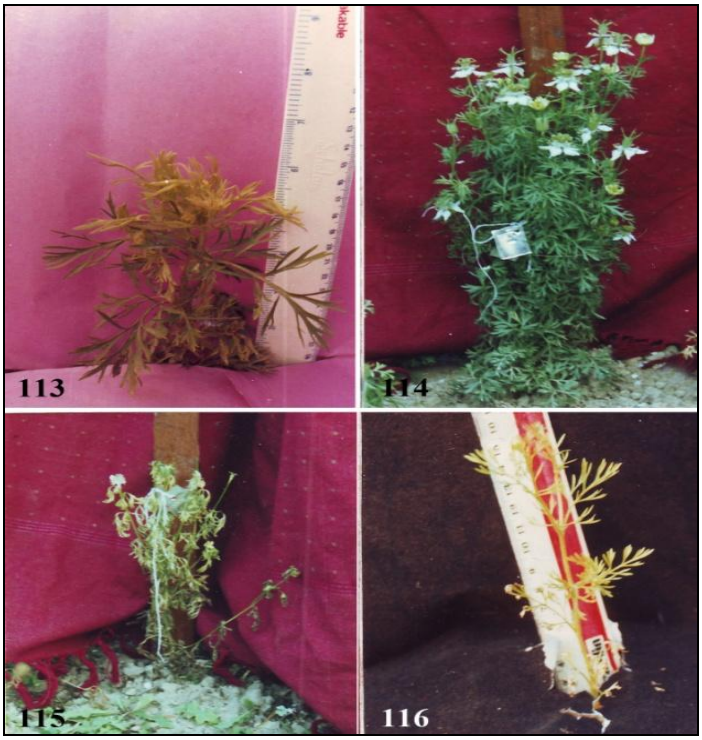
Figs. 92-97. Meiotic configurations at MI (92, 94-97) and diplotene (93) in translocation heterozygotes. 92) 6II. 93)1IV+4II. 94) 1IV (chain, alternate) + 4II. 95) 1IV (chain, adjacent) + 4II. 96) 1IV (chain, alternate) + 4II. 97) 1IV (ring, alternate) + 4II. Bar=15 µm. [Source: Cytologia 67, 2002]



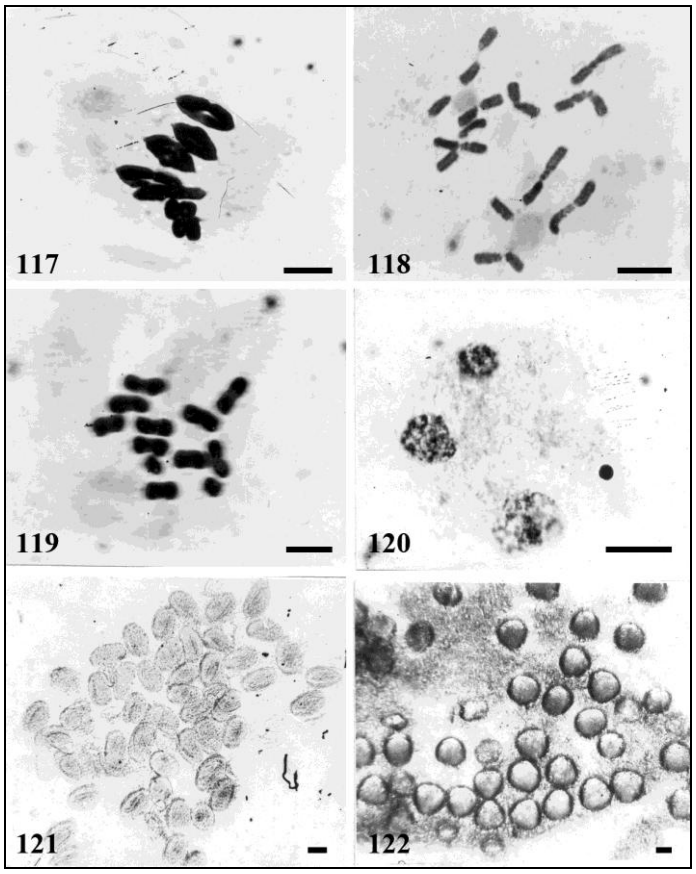
Figs. 98-103. Meiosis in translocate heterozygotes. 98 and 101) 1IV(ring, alternate) + 4II at MI. 99-100) 1IV (ring, adjacent) + 4II at MI. 102) 5-7 separation of chromosomes at AI. 103) Dicentric chromatid bridge with an acentric fragment at AI. Bar=15 µm. [Source: Cytologia 51, 1986]



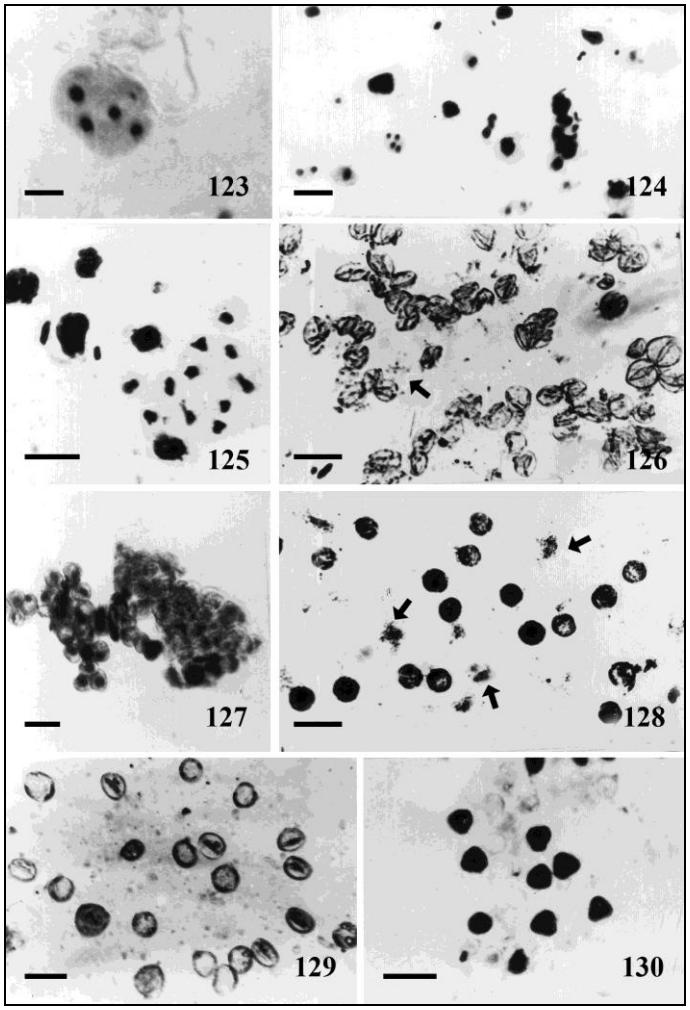
Figs. 104-112. Meiosis in synaptic mutants. 104-105) Early prophase I cells showing fuzzy chromosomes and lack of pairing. 106-112) MI chromosome associations. 106) 6II. 107) 5II+2I. 108-109) 4II+4I. 110) 2II+8I. 111) 1II+10I. 112) 12I. Bar=15 µm. [Source: Plant Archives 2, 2002]



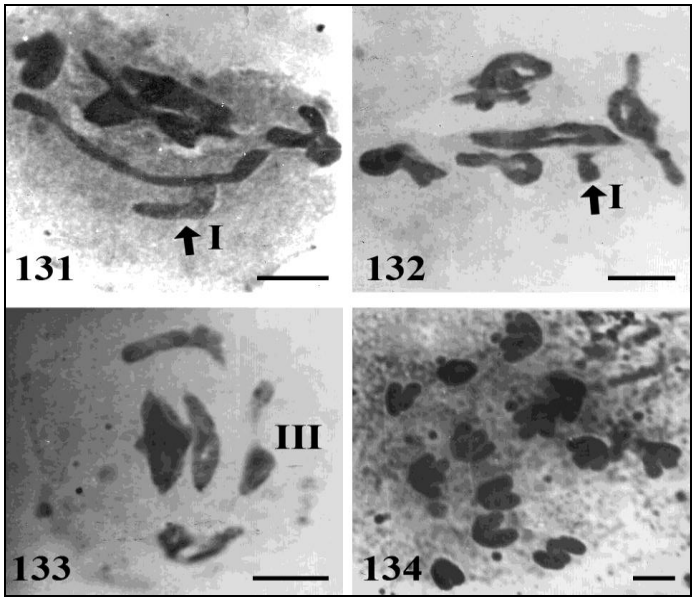
Figs. 113-116. Phenotype of male sterile mutants. 113) Mutants showing chlorophyll deficiency in pinnae of the apical part. 114) Bushy. 115) Crumpled pinnae. 116) Chlorophyll deficiency. [Source: Plant Archives 1, 2001]



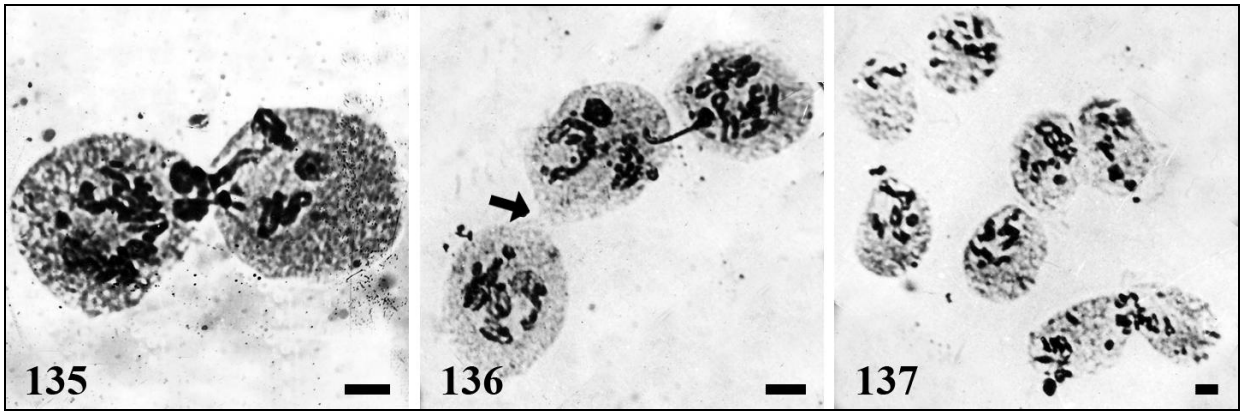
Figs. 117-122. Meiosis in a male sterile mutant (117-121). 117) 6II at MI. 118-119) 12I at MI. 120) AII with unequal spory (near complete degeneration of one pole). 121) Degenaration of microspores. 122) Fully stained round to oval shaped pollen grains in normal plants. Bar=15 µm. [Source: Plant Archives 2, 2002]



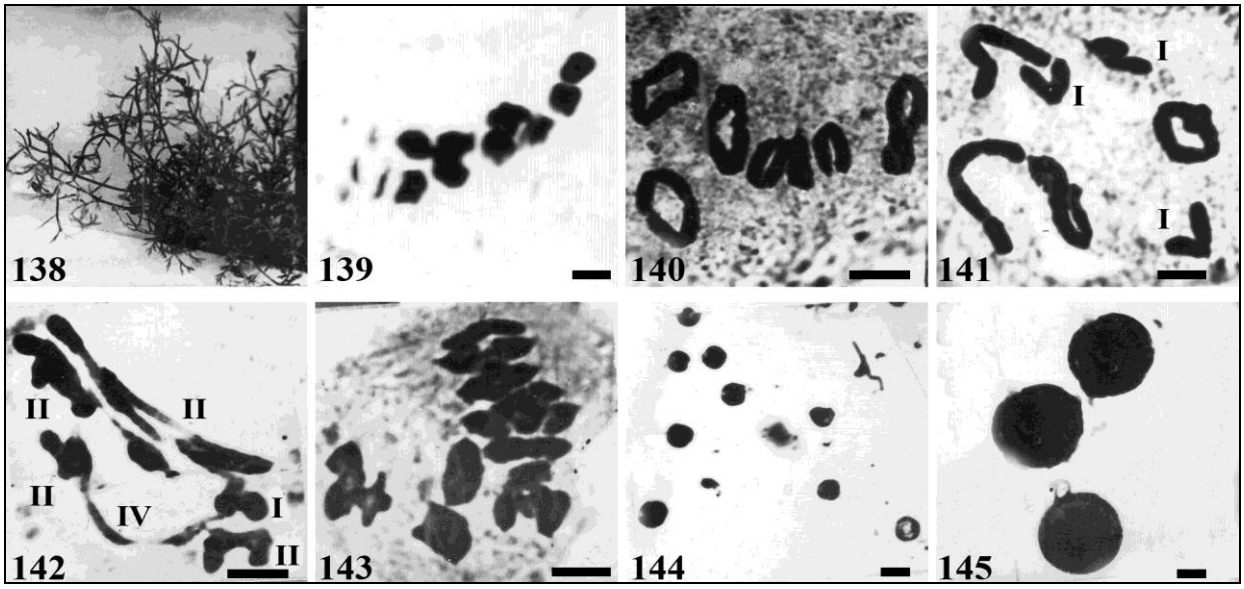
Figs. 123-130. Meiosis in male sterile mutants. 123-125) Agglutination of chromatin into unequal masses. 126 and 128) degenerative pollen grains. 127) Agglutinated pollen grains. 129) Small sized round unstained pollen grains. 130) Fertile pollen grains in normal plants. Bar=15 µm. [Source: Plant Archives 2, 2002]



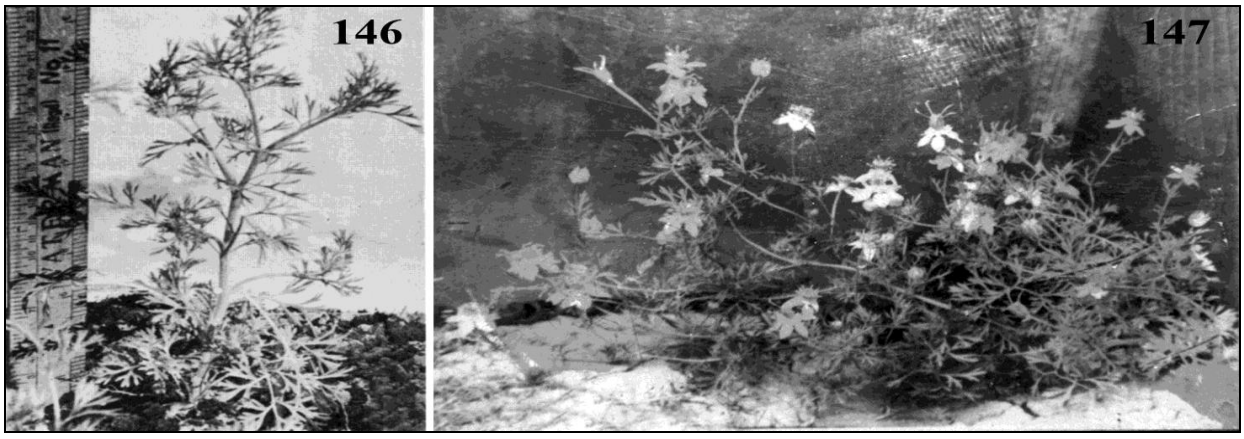
Figs. 131-134. Meiosis in a trisomic plant (2*n*= 13). 131-132) 6II+1I at MI. 133) 5II+ 1III at MI. 134) 9-7 separation of chromosomes at AI. Bar=15 µm. [Source: Cytologia 49, 1984]



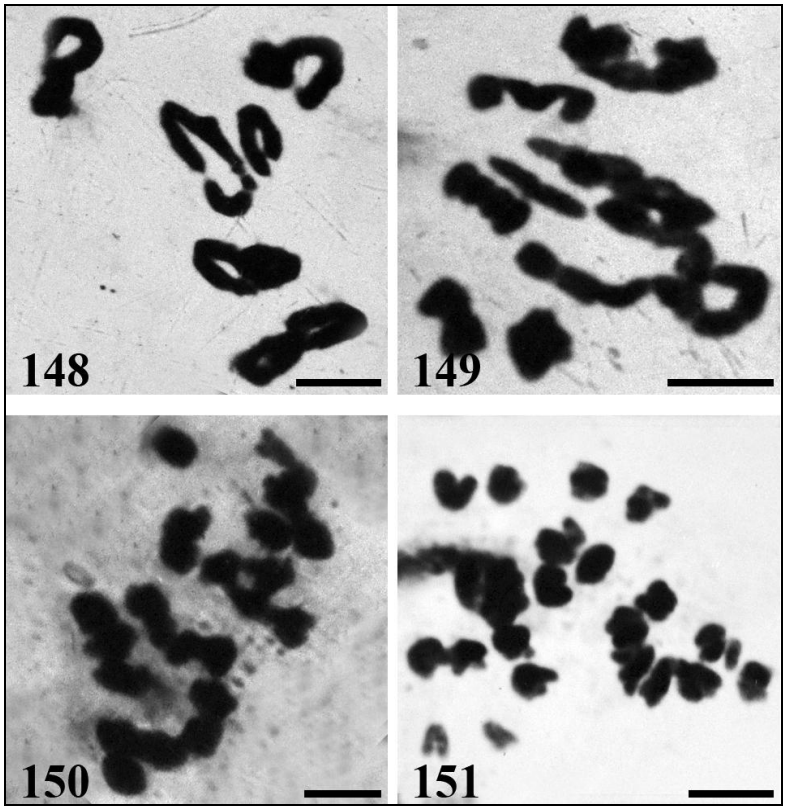
Figs. 135-137. Chromatin bridge (Fig. 136) and fusion of meiocytes (Figs. 135 and 137) in chromosome/ chromatin transfer. Bar=15 µm. [Source: Cytologia 49, 1984]



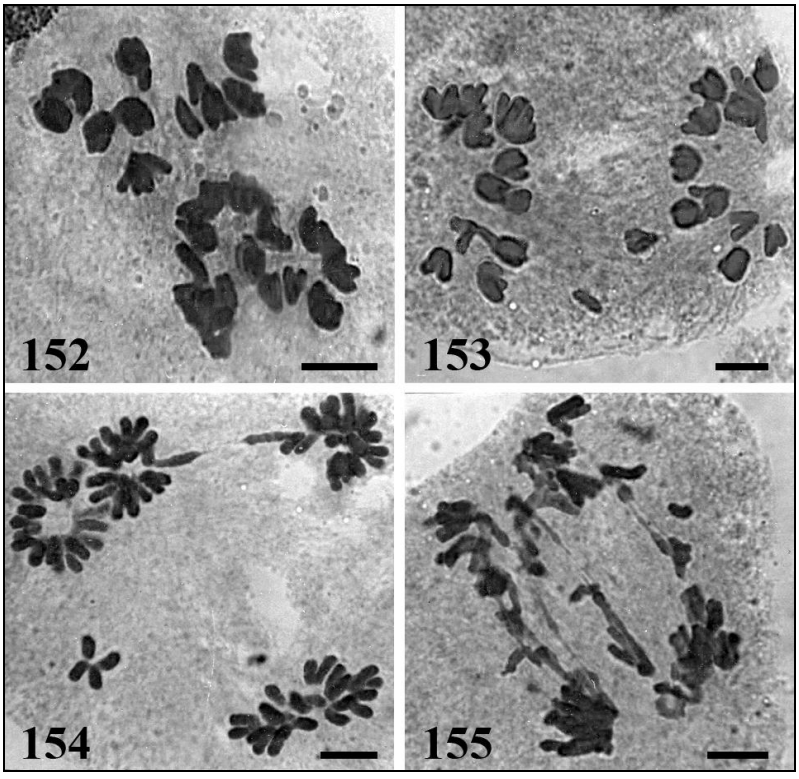
Figs. 138-145. 138) Aberrant plant showing lax branching nature and leaf deformity. 139-143. Meiosis in the aberrant plant. 139) 2II+5I (2*n*=9) at MI. 140) 6II (2*n*=12) at MI. 141) 4II+3I (2*n*=11) at MI. 142) 1IV+4II+1I (2*n*=13) at MI. 143) MI showing 12II (2*n*=24). 144-145. Pollen grains. 144) Stained and unstained small sized pollen grains in the aberrant plant. 145) Normal stained pollen grains in control. Bar=15 µm. [Source: Cytologia 50, 1985]



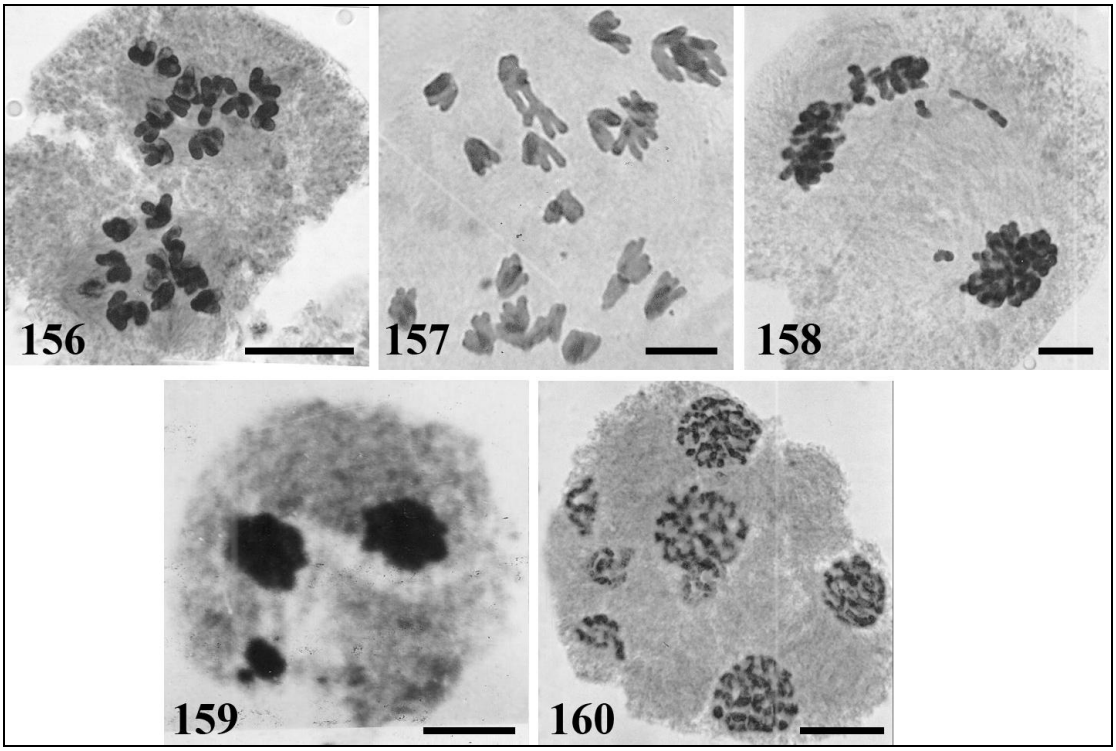
Figs. 146-147. 146) Normal diploid. 147) Autotetraploid showing synchronous flowering. [Source: Indian J. Genet. Plant Breed. 62, 2002]



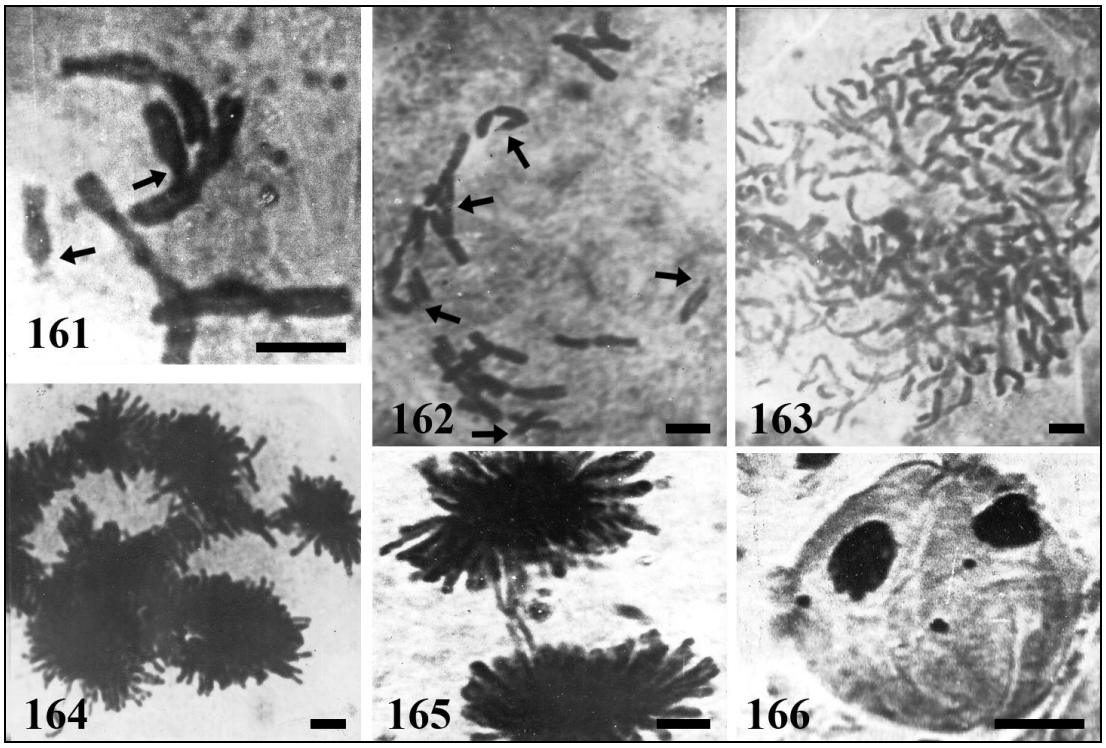
Figs. 148-151. Meiotic configuration at MI and AI (151). 148) 6II in diploid. 149-151. Meiosis in autotetraploid. 149) 1IV+9II+2I. 150) 2IV+4II+8I. 151) 8-16 separation of chromosomes. Bar=15 µm. [Source: Indian J. Genet. Plant Breed. 62, 2002]



Figs.152-155. AI configurations in the autotetraploid. 152) 11-13 separation. 153) 11-13 separation associated with a fragment. 154) Tripolarity along with a lagging chromosomes. 155) Multiple bridges with fragments. Bar=15 µm.



Figs. 156-160. AI (156-158) and AII (159-160) configurations in autotetraploids. 156-157) Unequal (11-13) separation of chromosomes. 158) Tripolarity with laggards. 159) Unequal spory. 160) Multiple spory. Bar=15 µm.



Figs. 161-166. Chromosome variations and abnormalities in callus tissue. 161) 2*n*=6. 162) 2*n*=24. 164-165) Enhanced ploidy level. 166) Bridge formation with higher ploidy. 167) Five extremely variable chromatin masses. Bar=15 µm.

CONCLUSION

Despite the major advancement of modern medicine in human health-care, it is still intangible and beyond reach to ailing humanity, especially the destitutes. In recent years plant based systems has been utilized for traditional medicine and phytotherapy. Medicinal plants are 'Gift of Nature' and *N. sativa* is one such plant with potential uses which can be explored for safe and effective herbal medicine for human benefit. Cytogenetical studies also revealed that the species can also be used as a model plant for better understanding of gene and chromosome relationship.

ACKNOWLEDGEMENT

This monograph is dedicated to those individuals who believe in herbal medicine and also to researchers working in the field of Cytogenetics.

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SEED BIOLOGY OF *ARTEMISIA MARITIMA* L. AN OVEREXPLOITED MEDICINALLY IMPORTANT SPECIES IN NORTH WEST HIMALAYAS

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Abstract: *Artemisia maritima* of family Compositae is an endangered perennial shrub with localized distribution because of its being highly habitat specific (Parihar *et al.*, 2011). Plants forming natural populations in Kishtwar Himalayas in J&K state, India, show high fruit and seed set in open fields (x =83.7%). Details of floral structure and events of floral biology reveal the species to be outcrossed, although it has the capacity to set seeds by selfing also. The same is accomplished through geitonogamy and by self pollen germinating at the point of nectary capping the ovary (Parihar *et al.* 2009).Seeds of the species, one per fruit , is with straight embryo and unique in being of two different colors , grey and brown. Both types are alike morphologically but differ in weight. On a moist filter paper, the %age of seeds germinating averages 34.33% for grey seeds and 47.5% for brown seeds. Most of the seedlings emerging out of these seeds however fail to establish. These observations reveal the manifestation of inbreeding depression in the species. This outcrossed species is supposedly forced to set seed by selfing due to squeezing of populations due to overexploitation and by a single individual occupying considerable area due to perennation for several years.

Keywords: *Artemisia maritima*, Seed

INTRODUCTION

Since last two decades, studies on reasons leading to squeezing of populations of some plant species have started bringing into focus several intrinsic problems too. The main among these are sluggish reproduction, inability to compete with other species of the community, prolonged seed-seed cycle, seedling mortality etc. Species that are rare and have small population size ,have been shown to suffer from inbreeding depression, also leading to decline in fitness. All these intrinsic factors have been cited to contribute heavily in making a species vulnerable to extinction (Frankel and Soule,1981; Levin,1983; Ledig,1986;Lacy,1992; Bruna and Kress,2002).

Artemisia maritima of Family Compositae has gained importance as the source of a drug called Santonin effective in its action on roundworms (Kaul, 1984). The species is used as an important fodder plant and has several other medicinal and aromatic uses also. All these above cited uses have helped on one hand in its enlistment as an important minor forest product in J&K state, but on the other hand have led to its overexploitation leading to considerable squeezing of its populations. This overexploitation has prompted some workers to place this species in endangered category (Kaul, 1996). The species is temperate with localized distribution. In J&K state, India it is restricted to Kishtwar Himalayas.

The plant perennates in winter via horizontal creeping root stock sprouting in the month of March. It remains in the vegetative state for 5-7 months and subsequently flowers in October/November when the day temperature fluctuates between 25°C and 30°C. Flowering is profuse and a single plant on an average produces 28,709±1964 capitula, each consisting of 6-13 homomorphic florets (Figure 1a).

Seed set is initiated during December and averages 83.4% on open pollination. Detailed studies on floral phenology and pollination strategies in this species suggest it to be predominantly cross pollinated but with the ability to produce seeds by geitonogamy also. Pollen germination at the point of nectary is also an interesting mechanism in this species and is responsible for some amount of seed set (Parihar *et al.* 2009). We observed fruit and seed set %age on open pollination and after unassisted selfing and also collected seeds from plants growing in district Kishtwar of J&K state during 2005-2009. The present communication deals with interesting observations made on some aspects of seed biology of this species.

MATERIAL AND METHOD

Populations of *Artemisia maritima* L. were identified and tagged in four different localities in Kishtwar town of J&K state. Individual plants were tagged at each place for observations on plant morphology, flowering phenology and fruit and seed set.

Fruit and seed set

Fruit and seed set on open pollination were observed in plants growing open in the field. Number of flowers/capitulum, number of fruits set/capitulum and seed set/ capitulum were counted for these plants.

%age fruit and seed set on open pollination was calculated as under:

$$\%\text{age fruit / seed set / inflorescence} = \frac{\text{Total furit/seed count/inflorescence}}{\text{Total number of flowers/inflorescence}} \times 100$$

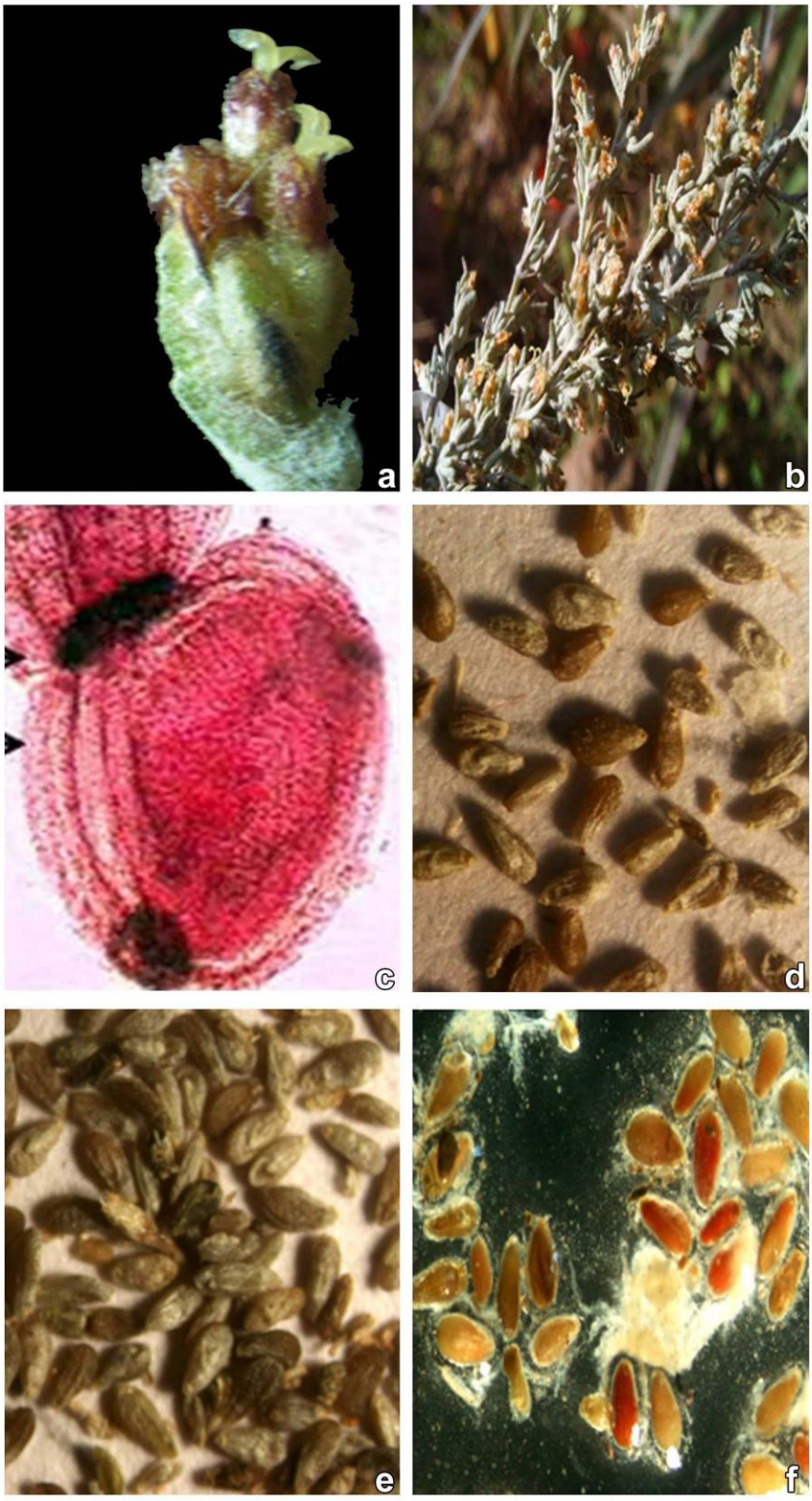

Figure 1a-f - a. A Capitulum of *Artemisia maritima* bearing florets with bifid extruding stigmas. b. Axillary spikes of *Artemisia maritima* bearing floral heads. c. An epigynous nectary capping one ovuled ovary at the base of style. d-e. Brown and grey seeds of *Artemisia maritima* f. Seed viability as revealed by TTC(2,3,5-triphenyl tetrazolium chloride) test.

Bagging experiment

Single, 3-6 inflorescences together and whole inflorescence branches were bagged on different plants with butter paper bags of appropriate size. These were later checked for fruit and seed set by geitonogamous pollination using the formula as elaborated for open pollination. Stigmas were decapitated in some florets as soon as they came out. These florets were then bagged. Data on fruit and seed set were then recorded for these inflorescences and also for individual flowers in these inflorescences to check fruit set by pollen germinating at the point of nectary.

Seed viability and seedling establishment

The viability of seeds was estimated by allowing seeds to germinate on a moist filter paper inside the petridishes kept at room temperature and in garden soil in experimental plots. Viability of seeds was also tested by 2,3,5-triphenyl tetrazolium chloride (TTC) test. For the same, seeds were placed in a petridish in TTC solution. The petridish was later placed in a humidity chamber and seeds were incubated in dark for 2-3 hours.

Seed weight and size

Seeds were kept in vials (n= 150) for determining weight and size. Seed size was determined using microscope equipped with ocular micrometer and seed weight was determined on electrical balance in laboratory.

RESULT AND DISCUSSION

Plants of *A. maritima* are perennial, perennating by horizontal rhizomes that sprout in the month of March to produce aerial offshoots. Each aerial offshoot attains a height of 40-71 cm. Flowering occurs after 5-7 months of vegetative phase. The flowers arise in axillary spikes terminating into heads (Figure1a). Single plant produces on an average 28,709±1964 capitula (Figure 1b) each of which is homomorphic and contains 6-13 bisexual florets (x= 8±2.0). Each floret contains five syngenecious stamens and a single pistil with bicarpellary syncarpous inferior ovary which is unilocular and one ovuled (Fig. 1c). Florets are protandrous, with anther dehiscence commencing 4-5 days before the onset of stigma receptivity.

Fruiting in *Artemisia maritima* initiates during early November and continues till early December. Fruit is cypsela; a dry indehiscent, one seeded fruit developed from a bicarpellary, syncarpous, inferior, unilocular ovary. Each fruit remains enclosed by persistent bracts. Fruit and seed set in open field is quite high and varies between 50-100%, it averages 83.4%. High investment in flower production and high seed set on open pollination portrays that *A. maritima* is reproductively efficient and has no apparent bottlenecks in its sexual reproduction. Data on unassisted pollination however reveals an interesting pattern.

Since individual flowers are too small to be checked for seed set on different pollination treatments, data on seed set was collected per inflorescence and different treatments were tried. Bagging of single inflorescence per branch on 25 different individuals resulted in low seed set varying between 11.1 to 22.2% (x = 16.50%). When three inflorescences were bagged together on 25 different individuals, seed set improved a bit but was still very low. It ranged from 16.6- 33.3% and averaged 25.71% .Random bagging of the six inflorescences of a branch together was done in 25 different individuals. Seed set on these inflorescences was still low and varied between 25-33% (x =30.62%). In 25 different individuals, single inflorescence was selected/ individual at random and in all the flowers of these inflorescences (x = 6.440±0.209), stigmas were decapitated as soon as they came out of the floret i.e. before these diverged their lobes. The seed set on these florets averaged x = 21.09±21.8. Except few seeds which were shrivelled and curved, all seeds resembled the ones set on open pollinated flowers.

Foregoing account reveals a reduction in seed set on unassisted selfing; the reduction being inversely proportional to the number of inflorescences bagged together. This suggests both inbreeding depression as well as availability of sufficient pollen as factors for this reduction. The data also clearly reveals that although *A. maritima* is cross-pollinated, it keeps a provision for self–pollination provided for some reasons, cross pollen transfer is not possible.

Seed one per fruit is brown or grey in colour (Figs. 1d and e). It bears a straight embryo and is small in size averaging 1.101x0.51 (mm). Seeds of both the colours i.e. grey and brown are similar in overall structure but differ in weight. The dry weight of brown seed averages 0.002 gm, while the dry weight of the grey seed is almost half (0.001gm). Seed viability was tested by 2,3,5-triphenyl tetrazolium trichloride (TTC) test. Ten samples of 50 seeds each were tested and an average of 28.20±1.17 turned pink on treating with TTC.The viability as tested by TTC test thus averaged 56.4% **(Fig. 1f)**.

Seed germination in *Artemisia maritima* takes 6-8 days under lab conditions. Seeds do not show any dormancy as they germinate without requiring any resting period. In experimental plots containing a mixture of sand and garden soil at Govt. Degree College, Kishtwar, seed germination averaged 60%. Out of 10 samples of 50 seeds sown, 30.8±1.9 showed the emergence of seedlings. More than 50% of these seedlings however failed to establish.

In another set of observations, grey and brown seeds were segregated into 6 heaps of 200 seeds each (3 grey and 3 brown) and their germination was

observed on a moist filter paper in the lab. Grey and Brown seeds showed significant differences in their germination %age. Germination of grey seeds averages 34.33% while that of brown seeds was more and averaged 47.5%. These thus showed apparent differences in their viability. Although displaying difference in color and viability, seeds in *Artemisia maritima* cannot be distinctively termed as of two different types as both resemble in size and overall morphology. This is unlike many other composites like *Rimerophotheca pluvialis*, *Felicia heterophyll*, *Chrysanthemum segetum*, *Calendula officinalis*, *Crepis dioscoridia* and *Syndrell noliflora* (Babcock,1947; Zohary, 1950; Levyna, 1972) where achenes display differences in being flat and winged as an adaptation for dispersal by wind.

All the above observations indicate that *A. maritima* has bottleneck in its life cycle at the point of seed germination. Although abundant investment is made in the production of enormous amount of seeds per plant, about half of this lot is viable and shows germination. Whether this hurdle is manifested again at the time of seedling establishment needs to be detailed out in lab condition, although inclination towards it is seen in field. Low seed germination as well as low seedling survival seems to cut down the high rewards of sexual reproduction in this species.

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REGENERATION STATUS AND SPECIES DIVERSITY ALONG THE FIRE GRADIENTS IN TROPICAL DECIDUOUS FOREST OF CHHATTISGARH

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Abstract: The present work aimed to study the impact or behavior of forest fire on regeneration status and diversity indices. Four sites were selected; in each of these sites pre-fire and post-fire observation were taken for measuring varying degree of disturbances. A total of 19 seedlings species were recorded during pre-fire season and 14 seedlings species were recorded during post-fire season, respectively. Along the fire gradients the tree species exhibited highest density of seedlings in low fire zone. It showed that non-fire zone contained more species as compared to burnt areas. The diversity pattern showed that the medium fire zone had maximum diversity followed by non-fire zone, whereas low fire zone had minimum Shannon index. Seedling density drastically reduced after post-fire (27.63%). In the high fire zone the seedling layer was much affected which will result discontinuation of conversion into sapling with the progress of time and ultimately the gap in the regeneration status.

Keywords: Diversity, Forest fire, Pre-fire, Post-fire, Regeneration

INTRODUCTION

Fire has been a part of the co-evolution of seasonally dry-forests and grasslands across the globe (Keeley and Bond, 1999**).** Forest fires cause enormous loss to the forest ecosystem, diversity of flora and fauna, and economic wealth. In India, out of 67.5 million ha of forests, about 55% of the forest cover is being annually subjected to fires (Gubbi, 2003). In accordance with positive attributes that fire enhances the productivity of ecosystems by releasing chemicals and nutrients locked up in the old herbage, but the uncontrolled fire destructs the micro-flora and micro-fauna in the top soil and litter layers in forests could have impacts on the organic decomposition and soil fertility (Kodandapani, 2001). Indian forests are burnt every summer, as it is believed to encourage the growth of succulent fresh grasses after the first rains. The forests are also burnt for collecting non-timber forest products, hunting and various other reasons. Very few studies that are available from Indian forests report that fires mostly affect ground vegetation. The findings of the present study will help to researcher, ecologist and foresters to work in other localities of the same area. Therefore, the present study was undertaken to investigate the impact of forest fire on regeneration status and diversity of different forest fire sites.

MATERIAL AND METHOD

The present study was conducted at Bhoramdeo Wildlife Sanctuary of Kawardha Forest Division in Chhattisgarh, Central India, after the repeated reconnaissance survey of Bhoramdeo Wildlife Sanctuary. The study area is located between 21^0 23'-22^0 00' North latitude and 80^0 58'-82^0 34' East longitude. The entire area of Bhoramdeo Wildlife Sanctuary is located in the Maikla Range of the Satpura hills. Total four sites (High, medium, low and non-fire zone) were selected; in each of these sites pre-fire and post-fire observation were taken for measuring varying degree of disturbances. The disturbance gradients (forest fire zones) were categorized by historical data taken from forest department.

The vegetation data were collected was analysed in different fire zones (i.e., high, medium, low and non-fire zone). A quadrat, of 5 x 5 m size was randomly laid for measuring seedling. The seedlings (<10 cm GBH) were measured at the collar height. Vegetational data were quantitatively analysed for frequency, density, abundance (Curtis and McIntosh, 1950). The relative frequency, relative density and relative basal area values were calculated following Phillips (1959). Regeneration status of species was totally based on population size of the seedlings and saplings (Khan *et al.*, 1987). Good regeneration if seedlings > saplings >adults; fair regeneration, if seedlings > or ≤ saplings ≤ adults; poor regeneration, if the species survives only in sapling stage, but no seedlings (saplings may be <,> or = adults). If a species is present only in adult form it is considered as not regenerating. Diversity indices were calculated following Sagar and Singh (1999).

RESULT AND DISCUSSION

During pre-fire in low and non-fire zone recorded similar number of seedling species (13 species) but the density was slightly higher (13680 individuals ha^{-1}) in low fire zone (Table 1) as compared to non-fire zone (12720 individuals ha^{-1}). The basal area also showed the similar trend. High fire zone recorded the lowest number of species (7) having the density of 10400 individuals ha^{-1}, whereas the medium fire zone showed a slight increase in species number (9) and density (10960 individuals ha^{-1}) as compared to high fire zone. The variability was noticed in the Shannon index from site to site in the study area of Bhoremdeo Wildlife Sanctuary. The seedling layer showed the highest Shannon index values recorded for medium

fire zone (2.80) followed by low fire zone (2.70), non-fire zone (2.47) and high fire zone (2.10). The Cd value was highest for high fire zone (0.29) followed by non-fire (0.25), low fire (0.22) and medium fire zone (0.17) whereas highest species richness values were recorded by low and non-fire zones (1.26 for both). The equitability (e) value was ranged from 0.96 to 1.27 across the fire zones. The lowest value of beta diversity was found under high fire zone. As far as the regeneration status concerned during pre-fire the species achieved 60% good regeneration under the low fire zone followed by non-fire, moderately fire and high fire zone. Few species was not regenerating at all (Table 3-7).

The total density of seedlings during post-fire season across the fire zones were ranged from 6800 to 10480 ha^{-1}. The high fire zone was recorded minimum density and number of species during both the season. Basal area value was drastically reduced after the fire as compare to pre-fire season. The value of Shannon index (Table 2) were ranged from 2.56 to 2.77, equitability 1.05 to 1.23, species richness 0.79 to 1.19, concentration of dominance 0.19 to 0.22 and beta diversity 1.16 to 1.75.

The high fire zone showed lower number of tree species as compared to non-fire zone. Kafle (2004) also reported that protected area (non-fire zone) also supports greater tree population as compared to fire affected areas. This might be happen due to repeated frequency and intensity of high fire disturbances during past whereas in medium and low fire zones, the number further declined as compared to non-fire zone therefore, the non-fire zone supported higher tree density. Kodandapani *et al.* (2008) have also reported the similar trend in his study while comparing the spatial, temporal and ecological characteristics of forest fires in the dry tropical ecosystem in the Western Ghats. Joshi (1990) observed higher values of seedling density on burned sites as compared to unburnt stand. The low fire zone supported highest number of seedlings ha^{-1} as compared to other fire zones. This result can be correlated to the effects of fire on juvenile die back. Several juvenile escaped from fire did not undergo stem die back, they exhibit height and growth patterns similar to unburnt seedlings (Saha, 2002). According to Kodandapani (2001) fire enhances the productivity of ecosystems by releasing chemicals and nutrients locked up in old herbages this results to regeneration of seedlings benefited from forest fire. The density values of seedlings and saplings are considered as regeneration potential of the species. The presence of good regeneration potential shows suitability of a species to the environment. Climatic factors and biotic interference influence the regeneration of different species in the vegetation across the different fire zones with varying degree of fire intensity and frequency. Higher seedling density values get reduced to sapling due to the biotic or anthropogenic disturbance and due to the competition for the resource utilizations. There are three major components which cause the success of regeneration of the tree species. These are the ability to initiate new seedlings, ability of seedlings and saplings to survive and the ability of seedlings and saplings to grow in the given site in a specific period of time (Good and Good, 1972).

Shannon index for seedling layer were ranged from 2.10 to 2.80, equitability 0.96 to 1.27, species richness 0.64 to 1.26, concentration of dominance 0.17 to 0.29 and beta diversity 1.46 to 2.71. This result also supports the findings made by Naidu and Sribasuki (1994) that young plants are more badly affected by fires than mature one. The lesser diversity in the frequent fire occurring dry deciduous forest leading to nonspecific forests, frequent fires could also lead to stands where most trees are even aged (Kodandapani 2001). Kafle (2004) reported that the protected area supported greater number of ground flora species. However, the burnt area contained higher species diversity and evenness indices than the protected area intotal.

CONCLUSION

The results on regeneration of tropical deciduous forests of the study area clearly demonstrated that density of seedlings as well as number of species decreased in high fire zone during pre-fire and post-fire season due to repeated frequency and high intensity of the fire in such area. In the high, medium and non-fire zone of post-fire season the regeneration of species also decreased, where as in low fire zone its density was increased due to reduction of competition and providing the clean bed to the growing ones. Due to severe fire, reduction of density may recorded in different gradient, if this type of anthropogenic pressure continues there is more threat to these forests in terms of species richness and there may be also possibility of invasion of exotic species to the fragile ecosystem. There is urgent need for management strategies to these forests is dependent on costs and ease at which they can be implemented and the benchmark we want to achieve. So as a first step, setting up an ecological reference level at which the landscape will be managed needs to be established based on scientific studies.

Table 1. Regeneration of species in different fire zones during the pre-fire and post-fire season in Bhoramdeo Wildlife Sanctuary.

Species	Pre-fire season				Post-fire season			
	High Fire Zone	Medium Fire Zone	Low Fire Zone	Non-Fire Zone	High Fire Zone	Medium Fire Zone	Low Fire Zone	Non Fire Zone
Anogeissus latifolia Wall ex Bedd.	--	+	+	+	+	+	+	+
Buchanania lanzan Spreng.	+	--	+	+	+	+	+	+
Butea monosperma (Lamk) Taub.	--	--	--	--	+	--	--	--
Casearia graveolens Dalz.	+	+	+	+	--	--	--	--
Cassia fistula Linn.	--	+	--	+	+	+	+	+
Chloroxylon swietenia D.C.	--	+	--	--	--	--	--	--
Dalbergia paniculata Roxb.	--	+	--	--	--	--	--	--
Diospyros melanoxylon Roxb.	+	+	+	+	+	+	+	+
Grewia tiliaefolia Vahl.	--	--	--	--	--	+	+	+
Kydia calycina Roxb.	--	+	+	--	--	--	--	--
Lagerstroemia parviflora Roxb.	+	+	+	+	--	+	+	+
Mitragyna parviflora (Roxb.) Korth.	--	--	--	+	--	--	--	--
Ougeinia oojeinensis (Roxb.) Hochr.	--	+	+	+	--	+	+	+
Emblica officinalis Gaertn	--	--	+	--	--	+	+	+
Saccopetalum tomentosum (H F.) Thoms	--	--	--	+	--	--	--	--
Schleichera oleosa (Lour.) Oken	--	--	+	+	--	--	--	--
Shorea robusta Gaertn.f.	+	--	+	+	+	--	+	+
Sterculia urens Roxb.	--	--	+	--	--	+	--	--
Syzygium cumini (Linn.) Skeels.	+	--	+	--	+	--	+	+
Terminalia alata Heyne ex Roth.	+	--	--	+	+	+	+	+
Terminalia chebula Retz.	--	--	+	+	--	--	--	+

+ indicating presence of the species whereas -- indicating absent of the species in different fire zone

Table 2. Comparisons of community characters of different forest fire zones of Bhoramdeo Wildlife Sanctuary during pre-fire and post-fire season

Pre-fire season					
Vegetation Layer	Characters	High Fire Zone	Medium Fire Zone	Low Fire Zone	Non-Fire Zone
Seedling Layer	Species	7	9	13	13
	Density (individuals ha^{-1})	10400	10960	13680	12720
	Basal Area (m^2h^{-1})	0.48	1.81	2.25	1.86
	Shannon Index (H')	2.10	2.80	2.70	2.47
	Simpson's Index (Cd)	0.29	0.17	0.22	0.25
	Species richness (d)	0.64	0.86	1.26	1.26
	Equitability (e)	1.08	1.27	1.05	0.96
	Beta diversity (βd)	2.71	2.11	1.46	1.46
Post-fire season					
Vegetation Layer	Characters	High Fire Zone	Medium Fire Zone	Low Fire Zone	Non-Fire Zone
Seedling Layer	Species	8	10	11	12
	Density (individuals ha^{-1})	6800	7680	10480	9600
	Basal Area (m^2h^{-1})	0.477	0.560	0.562	0.70
	Shannon Index (H')	2.56	2.77	2.65	2.62

	Simpson's Index (Cd)	0.20	0.19	0.21	0.22
	Species richness (d)	0.79	1	1.08	1.19
	Equitability (e)	1.23	1.20	1.10	1.05
	Beta diversity (βd)	1.75	1.4	1.27	1.16

Table 3. Regeneration status of tree species in High Fire Zone of Bhoramdeo Wildlife Sanctuary

Species	**Seedlings ha^{-1}**	**Saplings ha^{-1}**	**Trees ha^{-1}**	**Status**
Adina cordifolia Hook.f.	--	--	15	Not regenerating
Anogeissus latifolia Wall ex Bedd.	--	20	10	Poor regeneration
Bridelia retusa (Linn.) Spreng.	--	--	5	Not regenerating
Buchanania lanzan Spreng.	320	15	5	Good regeneration
Butea monosperma (Lamk) Taub.	--	5	--	Poor regeneration
Casearia graveolens Dalz.	720	--	--	Fair regeneration
Diospyros melanoxylon Roxb.	3360	55	--	Good regeneration
Grewia tiliaefolia Vahl.	--	5	--	Poor regeneration
Lagerstroemia parviflora Roxb.	560	15	--	Good regeneration
Lannea coromandelica (Houtt.) Merr.	--	--	15	Not regenerating
Ougeinia oojeinensis (Roxb.) Hochr.	--	10	40	Poor regeneration
Emblica officinalis Gaertn	--	30	--	Poor regeneration
Schleichera oleosa (Lour.) Oken	--	10	15	Poor regeneration
Shorea robusta Gaertn.f.	4400	90	110	Good regeneration
Sterculia urens Roxb.	--	20	5	Poor regeneration
Syzygium cumini (Linn.) Skeels.	800	5	--	Good regeneration
Terminalia alata Heyne ex Roth.	240	15	35	Good regeneration
Terminalia chebula Retz.	--	10	--	Poor regeneration

Table 4. Regeneration status of tree species in Medium Fire Zone of Bhoramdeo Wildlife Sanctuary

Species	**Seedlings ha^{-1}**	**Saplings ha^{-1}**	**Trees ha^{-1}**	**Status**
Adina cordifolia Hook.f.	--	--	5	Not regenerating
Anogeissus latifolia Wall ex Bedd.	720	60	60	Good regeneration
Boswellia serrata Roxb. ex Colebr.	--	5	25	Poor regeneration
Buchanania lanzan Spreng.	--	--	15	Not regenerating
Careya arborea Roxb.	--	--	10	Not regenerating
Casearia graveolens Dalz.	1440	155	25	Good regeneration
Cassia fistula Linn.	880	5	--	Good regeneration
Chloroxylon swietenia D.C.	320	--	--	Fair regeneration
Dalbergia paniculata Roxb.	240	25	15	Good regeneration
Diospyros melanoxylon Roxb.	2800	80	10	Good regeneration
Kydia calycina Roxb.	800	35	10	Good regeneration
Lagerstroemia parviflora Roxb.	2720	105	30	Good regeneration
Lannea coromandelica (Houtt.) Merr.	--	20	55	Poor regeneration
Ougeinia oojeinensis (Roxb.) Hochr.	1040	55	65	Good regeneration
Emblica officinalis Gaertn	--	5	5	Poor regeneration
Semecarpus anacardium L.	--	15	15	Poor regeneration
Sterculia urens Roxb.	--	25	15	Poor regeneration

Table 5. Regeneration status of tree species in Low Fire Zone of Bhoramdeo Wildlife Sanctuary

Species	**Seedlings ha^{-1}**	**Saplings ha^{-1}**	**Trees ha^{-1}**	**Status**
Adina cordifolia Hook.f.	--	10	15	Poor regeneration
Aegle marmelos Linn.	--	--	5	Not regenerating
Anogeissus latifolia Wall ex Bedd.	400	20	--	Good regeneration

Buchanania lanzan Spreng.	640	110	10	Good regeneration
Butea monosperma (Lamk) Taub.	--	10	--	Poor regeneration
Casearia graveolens Dalz.	2640	10	--	Good regeneration
Cassia fistula Linn.	--	10	10	Poor regeneration
Diospyros melanoxylon Roxb.	1440	60	--	Good regeneration
Kydia calycina Roxb.	560	25	--	Good regeneration
Lagerstroemia parviflora Roxb.	1040	100	30	Good regeneration
Lannea coromandelica (Houtt.) Merr.	--	10	5	Poor regeneration
Ougeinia oojeinensis (Roxb.) Hochr.	80	15	40	Good regeneration
Emblica officinalis Gaertn	400	20	5	Good regeneration
Schleichera oleosa (Lour.) Oken	80	5	5	Good regeneration
Semecarpus anacardium L.	--	--	15	Not regenerating
Shorea robusta Gaertn.f.	5600	185	185	Good regeneration
Sterculia urens Roxb.	80	--	--	Fair regeneration
Syzygium cumini (Linn.) Skeels.	480	35	--	Good regeneration
Terminalia alata Heyne ex Roth.	--	15	15	Poor regeneration
Terminalia chebula Retz.	240	10	--	Good regeneration

Table 6. Regeneration status of tree species in Non-Fire Zone of Bhoramdeo Wildlife Sanctuary

Species	**Seedlings ha^{-1}**	**Saplings ha^{-1}**	**Trees ha^{-1}**	**Status**
Adina cordifolia Hook.f.	--	--	5	Not regenerating
Anogeissus latifolia Wall ex Bedd.	640	70	30	Good regeneration
Bombax ceiba Linn.	--	5	--	Poor regeneration
Bridelia retusa (Linn.) Spreng.	--	--	20	Not regenerating
Buchanania lanzan Spreng.	800	100	15	Good regeneration
Casearia graveolens Dalz.	1840	50	15	Good regeneration
Cassia fistula Linn.	80	--	10	Fair regeneration
Diospyros melanoxylon Roxb.	2640	75	--	Good regeneration
Garuga pinnata Roxb.	--	--	5	Not regenerating
Grewia tiliaefolia Vahl.	--	10	5	Poor regeneration
Kydia calycina Roxb.	--	20	5	Poor regeneration
Lagerstroemia parviflora Roxb.	80	60	5	Good regeneration
Lannea coromandelica (Houtt.) Merr.	--	10	25	Poor regeneration
Madhuca longifolia Roxb.	--	--	5	Not regenerating
Mitragyna parviflora (Roxb.) Korth.	480	25	15	Good regeneration
Ougeinia oojeinensis (Roxb.) Hochr.	80	25	70	Good regeneration
Emblica officinalis Gaertn	--	15	--	Poor regeneration
Saccopetalum tomentosum (H F.) Thoms	80	10	15	Good regeneration
Schleichera oleosa (Lour.) Oken	400	10	5	Good regeneration
Shorea robusta Gaertn.f.	5440	465	345	Good regeneration
Sterculia urens Roxb.	--	5	5	Poor regeneration
Terminalia alata Heyne ex Roth.	80	20	20	Good regeneration
Terminalia chebula Retz.	80	15	10	Good regeneration

Table 7. Regeneration status of species in different fire zones of Bhoramdeo Wildlife Sanctuary

Fire Zones	**Regeneration status (in percentage)**			
	Good regeneration	**Fair regeneration**	**Poor regeneration**	**Not regenerating**
High Fire Zone	33.33	5.55	44.44	16.66
Medium Fire Zone	47.06	5.88	29.41	17.65
Low Fire Zone	60	5	25	10
Non-Fire Zone	52.17	4.35	26.09	17.40

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TREE LAYER COMPOSITION AND CARBON CONTENT OF OAK AND PINE IN LOHAGHAT FORESTS OF KUMAUN HIMALAYA

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Abstract: Present study deals with composition of tree species, biomass and carbon content of forests in Lohaghat (Champawat) in Kumaun Himalaya. Total 06 tree species were reported from the study forest sites i.e. *Quercus leucotrichophora, Pinus roxburghii, Cedrus deodara, Myrica esculenta, Prunus cerasoides and Xanthoxylum alatum*. The *Quercus leucotrichophora* was dominant tree (82.7%) in the study forest site. Oak tree shared maximum basal area ($24.96 m^2 ha^{-1}$) and important value index (210.72). Total density of trees, seedlings and saplings was 2860 ind ha^{-1}. Of this, tree, seedling and sapling shared 46.5, 21.0 and 32.5 percent. The biomass and carbon content of oak and pine was 128.10 t ha^{-1} and 72.87 t ha^{-1}, respectively. Of these, oak trees shared 79.19 % biomass and 81.5 % carbon, respectively. The findings of density, basal area, biomass and carbon content depicted that forest is in young stage with less number of tree species, needs a proper management and conservation so that tree layer species composition, biomass and carbon stocks could be increased.

Keywords: Basal area, Biomass, Carbon content, Density, Tree species

INTRODUCTION

Forests play a significant role in economy and ecology of any region, state and country in the world as they are one of the major natural resources covering 3952 million hectares (nearly 4 billion ha), which accounted for about 30 percent. Of this, natural forests and plantations accounted for about 95 and 05 percent, respectively. The rate of increase of forest plantation area is 2.8 million hectares per year. The carbon stocks in forest biomass are 283 Giga tons (Gt) but it is decreasing at the rate of 1.1 Gt per year. India still accounted for 23.41% forests area however; forest area should be at least $1/3^{rd}$ (33.3%) of the total geographical area of country as per the national forest policy.

Forest area in Uttarakhand accounted for about 3.5 million hectares (65 %) of the state's geographical area (FSI, 2009). However, the forest cover is about 45% of its forest area. The growing population pressure on forests for their basic needs such as wood, fuel, fodder and other forest products has created a serious concern about the sustainability of forest ecosystem. Thus the delicate relationship between man and forest has shattered, which requires a concrete policy for management and development of forest in the Himalaya region. In Uttarakhand, Sal(*Shorea robusta* Gaertn. F.) and Chir-pine(*Pinus roxburghii*) are the dominated tree species in forests occurred upto 1000m and 1200-1800m elevation, respectively. Apart from these species, Banj oak (*Quercus leucotrichophora*) and other oak species i.e. Tilonj Oak (*Quercus floribunda*), Kharsu oak (*Quercus semecarpifolia*) and Rianj oak (*Quercus lanuginosa*) grow upto 1600-3000m. Beyond 3000m elevation the alpine scrubs and alpine meadows are existed (Bisht and Lodhiyal, 2005). The forests and plantations in the Himalayan region were studied by Champion and Seth (1968), Ralhan *et al* (1982), Tewari and Singh (1981; 1985), Saxena and Singh (1982 a; b), Saxena and Singh (1985), Rao and Singh (1985), Singh and Singh (1987), Chaturvedi and Singh (1987), Rawat and Singh (1988), Adhikari *et al* (1991), Lodhiyal (2000) and Lodhiyal *et al* (2002) and Lodhiyal and Lodhiyal(2003). The structure and function of plantations such as poplar and eucalyptus studied by Lodhiyal (1990), Bargali *et al* (1992), Lodhiyal *et al* (1995) and Lodhiyal and Lodhiyal (1997).

Population structure of a species in forest can convey partly its regeneration behaviors, in relation to the reproductive strategy. Importance is given to the number of saplings under adult tree for predicting future comparison of a forest community (Singh and Singh, 1992). Saxena and Singh (1982) have analyzed the size class distribution of major species in several forest types of Central Himalaya. However, Singh and Singh (1992) recognized five patterns of population structure in Central Himalaya forests i.e. (i) The greater population of individuals in lower size classes than larger size classes indicates the frequent reproduction, (ii) The more number of individuals in middle size classes than lower to higher classes means the population in on the way to extinction, (iii) The lower age of seedlings than saplings means the fair reproduction in the past but the continuation of reproduction at the lower rate, (iv) The occurrence of seedlings and saplings or saplings only other than dominant species may from a sub canopy species, (v) Absence of seedlings means, the species reproduced well earlier but at present the reproduction is stopped. The aim of forest ecologists is to understand dynamics of plant species of landscape particularly in relation to structure and function of forest ecosystem (Barnes *et al.,* 1998).

The over exploitation of forests in the central Himalayan region resulted a loss of biodiversity and species composition in their native habitats/sites. The impact of human influence on natural forests is so severe that the loss of biological pool due to

reduction of species diversity as well as it also leading towards the end of birth of forest species. The great loss is the loss of rich vegetation of the Himalayan region is increasing. In the recent years, Central Himalayan forest ecosystem witnesses the biotic disturbances. Such disturbances do not provide time for the ecosystem recovery and arrest the regeneration of important plant species in the forests of the region. This not only widens the gap but also changing the species composition of forests (Singh, 1998).

As far as biomass of forest is concerned, which is the main product of forests? It can be measured in the form of timber and non-timber products. How much biomass is stored in forest? Such information is important for every type of developmental needs of society, region and country. Biomass is estimated by the methods such as harvesting, chlorophyll, leaf area index, and remote sensing satellite data. In this study, we used the regression equation as developed by Rawat & Singh (1988) and Chaturvedi & Singh (1987).

As far as carbon storage and carbon sequestration is concerned, it is a burning issue of global warming and climate change, which become a global concern to mitigate the increasing concentration of green house gas specially the carbon dioxide gas in the atmosphere. How to combat this problem? Scientists are trying to evolve such a methods that could be reduced the excess amount of Co_2 from the atmosphere and to balance its equilibrium. In this regard, forest vegetation is considered as one of the best solution tool for mitigation of carbon concentration of the atmosphere. As forest play a significant role in sequestering the atmospheric Co_2 as well as act as a carbon sink.

Keeping this in view, we tried to estimate the carbon storage in the studied forests. The carbon stored both in living biomass (standing timber, branches, foliage/ leaves and roots) and in dead biomass (litter, woody debris, soil organic matter and other related forest products. Deforestation results in the loss of a major sink for carbon. However, any human activity that affects the amount of biomass in forest vegetation and soils has a potential either to sequester carbon from or to release carbon into the atmosphere.

One hectare of closed tropical forest can contain upto 220 tons of Carbon (t C), most of which when burnt, is released into atmosphere. However, one-hectare agroforestry established on deforested land in tropics perhaps as much as 2200 tC could be prevented from going into the atmosphere but it may be more or less productive because of varying forms of agroforestry. The natural regeneration in tropics stored 195 t C/ha over a period of 50 years is the highest rate of biomass productivity of natural ecosystem in the humid tropics. Afforestation in temperate latitude stored 120 t C/ha through high growth rates of plantations on the lands. Through agroforestry practice in tropics one-hectare land shows a medium value 95t C/ha. Such practice is also important from the standpoint of supporting the local populations. The reforestation practices in tropics and temperate latitudes have a medium carbon sequestration value 65 t C/ha and 56 t C/ha, respectively. Through carbon sequestration, the amount or rate of carbon accumulation could be increase by creating or enhancing carbon sinks through land use practices such as afforestation, reforestation and restoration of degraded lands, improved silvicultural techniques to increase growth rates and agroforestry practices. However, by carbon conservation strategy, we can reduce or prevent the rate of release of carbon already fixed in existing carbon sinks. It requires to conserve the biomass and soil carbon in the existing forests, By using the improved harvesting techniques that minimize the logging impacts, To improve the efficiency of wood processing, By using the effective fire protection measures and To use more effective burning carried out in both the forests and agricultural systems. The carbon substitution strategy will reduce the demand of fossil fuels by increasing use of wood either for durable wood products i.e. substitution of energy intensive material such as steel and concrete or for bio-fuel.

Thus, it is to conclude that the judicious forest management technique can contribute towards the emissions reduction and to carbon sequestration. The conservation of existing carbon stocks in forests is potentially a more powerful tool than carbon sequestration. However, forestry measures alone will not be enough to halt the increase in atmospheric Co_2 concentrations; thus, it requires various strategic tools and techniques with local people involvement in the resource conservation and management perspectives

The study objectives were: (i) to determine the density, frequency, abundance, A/F ratio tree species in forest site. (ii) to determine relative density, relative frequency, relative dominance and important value index (IVI) of tree species in forest and (iii) to calculate biomass production and carbon sequestration potential in forest.

MATERIAL AND METHOD

The study site lies between 29^0 24' N lat. And $79^0$28'E long. Of the total annual rainfall of 2000 mm about 75 % occurred in rainy season. The present study was carried out in the forests, located in Lohaghat, district Champawat, which fall between 1700 and 2000m elevation. The climate of entire study area is influenced by monsoon pattern of rainfall. On the basis of seasons, the whole year is divided mainly into rainy (mid-June-September), winter (November-February) and summer season (April-mid- June). There is a transitional period known as spring (March) and autumn (October), respectively. The temperature of Lohaghat was maximum (29.9^0C) in June and minimum (11.2^0C) in

January. The annual rainfall was 44.2 mm, which was maximum (11.6mm) in September (DST-U-Probe 2005). Geologically, the sites are consisting of sedimentary and metamorphic rocks consisting of sandstone, boulders, gneiss, alluvial with gravel, coarse and fine sand. The soil of the forests contains stones, gravels, sand, silt, and clay in different proportion. However, the colour of the soil varies from dark brown to reddish brown. The soils of the forests are mostly acidic in nature.

Quantitative analysis of vegetation

The quantitative information was carried out for density, frequency, abundance, A/F ratio, IVI (important value index) of tree layer composition of forest. The woody layer analysis was done using quadrat method of 10 x 10m size. Total 30 quadrats were placed randomly in each forest site during December 2009. The diameters of tree species at breast height (dbh at 1.37m) were measured with the help of tree Caliper. On the basis of field data, the tree density, frequency, abundance, A/F ratio and IVI were calculated based on the formulas as given by Density of tree species, the biomass of respective components of tree was estimated. Thereafter, by summing up of biomasses of each tree component, we determined the total forest tree biomass for each forest stand.

Carbon sequestration for each component of tree species *i.e.*, Banj oak, Tilonj Oak and Pine in each forest site was estimated. There is no information about the tree carbon in this forest area. Most of the carbon research has been described by researchers in many research journals and available literature suggested that forest carbon constitute between 45 to 50 percent of the dry matter (biomass) (Chan, 1982; Schlesinger, 1991) To estimate the carbon sequestration, we followed the methods as developed and mentioned by Magnussen and Read (2004) and Singh and Lodhiyal (2009), respectively. We have estimated the carbon sequestration simply by taking the fraction of biomass using the following formula: Carbon sequestration= biomass multiplied by factor as: C=B X 0.475

Curtis and McIntosh (1950) and Saxena and Singh, (1982). For quantitative analysis, forest tree species were divided into different diameter classes viz. seedlings were considered to be individuals 0-15cm (diameter at basal height), saplings, 15-30cm (diameter at breast height) and trees above 30cm Dbh ((diameter at breast height 1.37 m) as followed by Saxena *et al.* (1982).

Biomass and carbon allocation

For the estimation of tree biomass, the regression equations were used as developed by Rawat and Singh (1988) for banj oak (*Quercus leucotrichophora*,) and Chaturvedi and Singh (1987) for pine (*Pinus roxburghii*). The biomass of each component of tree species as bole, branch, twigs, foliage (leaves) and roots (stump root= the main root of tree having the small portion of stem with tape root), lateral roots= roots that aroused from the stump root part) and from roots=roots having lower diameter originated from the portion of lateral root) were calculated.

Where 'C' is the Carbon Content or Carbon sequestration potential and 'B' is the biomass (oven dry matter) of tree component. Thus summing up the carbon content of each tree component we, estimated the total carbon of tree species. The carbon of selected tree species was calculated by multiplying density of respective tree species. Carbon of all species was summed up to get total carbon sequestration potential of forest.

RESULT

Quantitative analysis of forest

The quantitative parameters of trees, seedlings and saplings of forests were studied. The data of each forest stand were collected by randomly placed 10 quadrats for seedlings, saplings and trees of different species in forest stand site is given in Table 1. The findings for each forest stand site are described below.

Table 1. Vegetation analysis of trees, seedlings and saplings of tree species in studied forest.

Tree species	Trees	Seedlings	Saplings
Oak (*Quercus leucotrichophora*)	1100	56	39
Pine(*Pinus roxburghii*)	70	03	09
Deodar(*Cedrus deodara*)	20	01	04
Kaphal(*Myrica esculenta*)	140	-	06
Paiya(*Prunus cerasoides*)	-	-	33
Timor(*Xanthoxylum alatum*)	-	-	02
Total	1330	60	93

Trees: The six (06) tree species viz., *Quercus leucotrichophora, Pinus roxburghii, Cedrus deodara, Myrica esculenta, Prunus cerasoides and Xanthoxylum alatum* were present. Total density of trees was 1100.0 trees ha^{-1}. The tree density ranged between 0.2 (*Cedrus deodara*) and 11.0 tree $100m^2$

(*Quercus leucotrichophora*). The frequency of tree species ranged between 10 and 100% and maximum for *Quercus leucotrichophora* and minimum for *Cedrus deodara* (Table 2). The abundance and A/F ratio ranged from 1.00 to 11 trees and 0.038 to 0.300. The A/F ratio showed that the species were distributed in all the patterns (Table 2).

Table 2. Density, frequency, abundance, A/F ratio and distribution pattern of tree species in studied forest stand in site.

Species	Category of layer	D (ind. ha^{-1})	F (%)	A	A/F ratio	DP
Oak	Trees	1100	100	11.00	0.110	Contagious
	Seedlings	560	90	6.22	0.069	Contagious
	Saplings	390	90	4.33	0.048	
Pine	Trees	70	50	1.40	0.028	Random
	Seedlings	30	10	3.00	0.300	Contagious
	Saplings	90	40	2.25	0.056	Contagious
Deodar	Trees	20	10	2.00	0.200	Contagious
	Seedlings	10	10	1.00	0.100	Contagious
	Saplings	40	20	2.00	0.100	Contagious
Kaphal	Trees	140	50	2.8	0.056	Contagious
	Seedlings	-	-	-	-	-
	Saplings	60	40	1.5	0.038	Random
Paiya	Trees	-	-	-	-	-
	Seedlings	-	-	-	-	-
	Saplings	330	90	3.66	0.041	Random
Timor	Tree	-	-	-	-	
	Seedlings	-	-	-	-	
	Saplings	20	10	2.00	0.200	Contagious

Note: D=Density, F=Frequency and A=Abundance; A/F ratio= Abundance of a species/frequency of same species dictates distribution pattern of a species and DP=distribution pattern. When A/F ratio value is < 0.025, means that the species is regularly distributed. When A/F ratio of a species ranges from 0.025 to 0.05, it means that species is randomly distributed. Whenever the A/F ratio is >0.05 and more means species are contagiously distributed (or clumped).

The total basal area of tree species was 36.76.59 cm^2 100 m^2 or 36.77 m^2 ha^{-1}. The basal area ranged from 36.66 to 2495.51 cm^2 100 m^2. It was maximum for Banj oak (*Quercus leucotrichophora*) and minimum for Paiya(*Prunus cerasoides*). The total IVI was reported 299.83 for all species occurring in forest site (Table 3). The most dominant species was *Quercus leucotrichophora* (IVI=210.72) in the site followed by Kaphal (*Myrica esculenta*) (IVI= 39.96) and Chir-Pine (*Pinus roxburghii*) (IVI= 35.48) (Table 3). The value for each species is mentioned in Table 3.

Table 3. Basal area, relative density, relative frequency, relative dominance and important value index of different tree species in studied forest stand.

Tree Species	BA (cm^2/100 m^2)	BA (m^{2}ha^{-1})	RD (%)	RF (%)	RD (%)	IVI
Quercus leucotrichophora	2495.51	24.95	82.7	46.45	81.57	210.72
Pinus roxburghii	320.75	3.21	5.26	22.72	10.5	35.48
Cedrus deodara	36.66	0.37	1.19	9.09	1.50	11.78
Myrica esculenta	823.88	8.24	10.52	22.72	6.72	39.96
	3676.50	36.77				299.94

Note: BA= Basal Area; RD= Relative density, RF= Relative frequency; RD=Relative dominance and IVI= Important value Index

Seedlings: The total 60 seedlings of four (04) tree species viz., *Quercus leucotrichophora, Pinus roxburghii, Cedrus deodar, and Myrica esculenta* were present. Seedlings of *Quercus leucotrichophora* were dominant in forest site. The total density of seedlings of all species was 60 ind.100 m^2 (6000 seedlings ha^{-1}). However, the individual density of seedlings among species ranged from 70 to 560 ind.100 m^2 (see Table 2). The frequency of tree species ranged between 10 and 90% and maximum for *Quercus leucotrichophora* and *Myrica esculenta*

(see Table 2). However, abundance and A/F ratio of seedlings ranged from 1.00 to 6.22 individual $ha^{-1}$and 0.069 to 0.300, respectively (see Table 2). The **IVI** of seedlings was ranged from 9.13 to 155.67 for the tree species seedlings (Table 4).

Table 4. Relative density, relative frequency, relative dominance and important value index of seedlings of different species in studied forest stand site.

Seedlings of tree Species	RD(%)	RF(%)	RD(%)	IVI
Quercus leucotrichophora	76.71	64.28	24.68	165.67
Pinus roxburghii	6.84	7.14	1.98	15.96
Cedrus deodara	1.37	7.14	0.62	9.13
Prunus cerasoides	15.06	21.42	72.73	109.21
Total				299.97

Note: RD= Relative density, RF= Relative frequency; RD=Relative dominance and IVI= Important value Index

Saplings: The total 93 saplings of six (06) tree species i.e. *Quercus leucotrichophora, Quercus floribunda, Pinus roxburghii, Cedrus deodara, Myrica esculenta and Prunus cerasoides* were present in forest stand site. The density ranged from 0.2 (Timor) to 3.9 ind$100m^2$(Banj oak). However, the total density of saplings was 9.3 individual 100 m^2 or 930 ind ha^{-1}(see Table 2). The IVI of saplings of *Quercus leucotrichophora* was maximum (130.77.67) followed by *Xanthoxylum alatum* (20.06) (Table 5).

Table 5. Relative density, relative frequency, relative dominance and important value index of saplings of different species in studied forest stand in site.

Seedlings of tree Species	RD (%)	RF (%)	RD (%)	IVI
Quercus leucotrichophora	47.56	31.03	52.18	130.77
Pinus roxburghii	10.97	13.79	4.59	29.35
Cedrus deodara	4.87	13.79	3.67	22.33
Myrica esculenta	7.31	13.79	12.08	33.18
Prunus cerasoides	26.82	24.13	13.28	64.23
Xanthoxylum alatum	2.44	3.45	14.17	20.06
Total				299.92

Note: BA= Basal Area; RD= Relative density, RF= Relative frequency; RD=Relative dominance and IVI= Important value Index

Tree biomass and carbon storage: The total number of Banj-oak (*Quercus leucotrichophora*) trees was 133 in studied forest. Of this, Oak, pine, deodar and Kaphal was 110, 07, 02 and 14, respectively. The average diameter of oak and Pine trees was 17.07 and 24.0cm.

Tree biomass: Total biomass of Banj-oak trees was 1014.55.73 kg /$100m^2$ or 101.45 tone ha^{-1}. Of this, maximum (25.30 t ha^{-1}) and minimum (0.81 t ha^{-1}) biomass was shared by bole and fine roots, respectively (Table 6). However, the biomass of Chir-pine was 266.53 kg/$100m^2$ or 26.65 t ha^{-1}. The maximum (8.16 t ha^{-1}) and minimum (1.53t ha^{-1}) shared by branch and cone component, respectively (Table 7).

Table 6. Biomass and carbon content of Banj oak (*Quercus leucotrichophora*) tree species in forest stand site.

Components	Biomass (Kg in $100m^2$)	Biomass (t ha^{-1})	CSP (Kg $100m^2$)	Carbon (t ha^{-1})
Bole	253.31	25.3	120.30	12.03
Branch	238.83	23.8	113.40	11.34
Twig	168.92	16.9	80.24	8.02
Foliage	169.99	16.9	80.75	8.07
Stump root	189.25	18.9	89.89	8.99
Lateral roots	149.88	14.9	71.19	7.12
Fine roots	80.81	0.81	38.38	3.84
Total	1014.55	101.45	594.15	59.41

Note: CSP = Carbon sequestration potential

Carbon storage: The total Carbon content of Banj oak trees was 594.15 kg /$100m^2$ or 59.41 t ha^{-1}. Of

this, bole accounted for maximum (12.30 t ha^{-1}) (Table 6). However, total carbon storage in Chir-pine was 134.56kg /100m^2 or 13.46 t ha^{-1}. Of which branch accounted for maximum 4.68 t ha^{-1}(Table 7).

Table 7. Biomass and carbon content of Chir-pine (*Pinus roxburghii*) tree species in forest stand site.

Components	**Diameter (cm)**	**Density trees /100m^2**	**Biomass (Kg /100m^2)**	**Biomass (t ha^{-1})**	**CSP (Kg/100m^2)**	**Carbon (t ha^{-1})**
Bole	24.20	0.7	39.51	3.95	18.77	1.88
Branch: I order	24.20	0.7	43.56	4.36	28.69	2.87
Branch: II order	24.20	0.7	38.01	3.80	18.05	1.81
Foliage	24.20	0.7	27.43	2.74	13.03	1.30
Cone	24.20	0.7	15.34	1.53	7.29	0.73
Stump root	24.20	0.7	36.41	3.64	17.29	1.73
Lateral roots	24.20	0.7	37.51	3.75	17.82	1.78
Fine roots	24.20	0.7	28.76	2.88	13.62	1.36
Total			266.53	26.65	134.56	13.46

Note: CSP =Carbon sequestration potential

DISCUSSION AND CONCLUSION

Forests of Himalaya play a significant role in sustainable development of society and conservation of resources. They not only protect and conserve the soil, water and biodiversity of the hills regions but also have a very vital role in the development of society and watershed management. Forests provide the timber and non-timber products and also protect environment polluted and degraded by human activities. Plant diversity has affected by various climatic, edaphic, topographic and biotic pressures such as animals and human beings that are carried out in forest ecosystem. The sustainability of forest ecosystem can be determined and assessed on the basis of presence, structure and function of existing plant species therein. Himalayan ecosystems are facing many problems that are associated directly and influencing the conservation and development of natural resources like soil, water land, forest and biodiversity in the regions. Present study was basically to know the tree layer composition in selected forest. The vegetation analysis was carried out for trees, seedlings and saplings existing in the forest. For the analysis of forest, first, we divided the whole forest into three forest sites. In each forest stand site, we studied, density, frequency, abundance, A/F ratio, distribution pattern of each vegetation category i.e. trees, seedlings and saplings. Apart from these, the basal area, relative density, relative frequency, relative dominance, important value index (IVI), tree biomass and carbon sequestration of trees were also determined in each forest site. The data presented in each table and in the text are the average of each forest site.

All the above parameters were studied by using quadrat method, regression equations for tree species as given by the earlier researchers as mentioned in materials and methods. The carbon sequestration for Banj-oak and Chir-pine tree species was studied in each selected forest site. The average findings of studied forest sites are given in Table 8.

Table 8. Comparative accounts of important vegetation parameters of forest stand in studied sites are given here.

S. No.	**Parameters**	**Results**
1.	Density (ind.ha^{-1})	
	Forest trees	1330(46.5)
	Seedlings	600(21.0)
	Saplings	930(32.5)
	Total	2860(100)
2.	Basal area (m^2ha^{-1})	
	Oak tree	36.77(60.0)
	Pine tree	24.95(40.0)
	Total	61.72(100.)
4	IVI	
	Oak trees	299.94(210.72)
	Seedlings	299.97(165.67)
	Saplings	299.92(130.77)

5	Tree biomass (tha^{-1})	
	Banj oak tree	101.45(79.19)
	Chir-pine tree	26.65(20.80)
	Total	128.10(100.0)
6	Carbon storage (t ha^{-1})	
	Banj tree	59.41(81.5)
	Pine tree	13.46(18.5)
	Total	72.87(100.0)

Note: In parenthesis the value of oak and pine species are given, which showed the maximum shared among the associated species.

The vegetation parameters of studied forest site are mentioned in Table 8. Total density of tree layer was 2860 ind. ha^{-1}. Of this, tree, was higher in both forest site as compare to seedlings and saplings. The maximum basal area (60%) was shared by oak trees. The important value index (IVI) of Banj-oak tree species shared maximum followed by its seedlings and saplings in forest (Table 8).

The total biomass of tree species was 132.12 t ha^{-1}. The maximum biomass of forest was shared by *Quercus leucotrichophora* and followed by Chir-pine trees (Table 8). Present finding of density of banj oak trees, seedlings and saplings are on higher side than the values reported for Banj -oak tree, seedlings and saplings in reserve forest of Nainital forest division i.e. 43-170 trees, 323-1200 seedlings and 123-500 saplings per ha^{-1}(Swati and Lodhiyal, 2005). Our findings are on higher side than the value reported for oak forest of central Himalayan region (Rawat and Singh, 1988). The values reported for pine trees in present study are lower side than the values of pine forests studied by Chaturvedi and Singh (1987). However, the values of biomass of oak and pine are on lower side than the values of oak forests studied by Rawat and Singh (1988) and Chaturvedi and Singh (1987). However, there is no information about carbon sequestration potential of the present studied forests. The carbon sequestration was 72.87 t ha^{-1} in studied forest. In the context of global warming and climate change processes, vegetation has significant role in mitigation of carbon through forest carbon sink. Thus it is imperative to know that what type of stand structure, composition, species diversity, biomass and carbon potential existing in forests occurring in different geographical locations. It is concluded that present findings provide the quantitative information of forest. These findings would be useful in conservation and management strategies in the Himalayan forest ecosystems as well as researchers working in the ecological and developmental aspects of forests and community.

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MORPHOLOGICAL AND CYTOLOGICAL STUDIES IN *NIGELLA SATIVA* L. AND *N. DAMASCENA* L. (RANUNCULACEAE)

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Abstract: *Nigella sativa* L. (black cumin; potential herb with immense therapeutic uses apart from its spice yielding property; cultivated variety – *Persian Jewels*) and *Nigella damascena* L. (commonly known as 'love-in-a-mist', grown in gardens throughout temperate region of the world; cultivated variety – *Miss Jekyll blue* obtained from Sutton and Sons', Kolkata and an accession 0016287 obtained from Royal Botanical Garden, Kew, London) members of the family Ranunculaceae were grown in the Experimental field plots of Department of Botany, Kalyani University (Nadia, West Bengal plains, latitude 22°50′ to 24°11′ N, longitude 88°09′ to 88°48′ E, elevation 48 ft. above sea level, sandy loamy soil) for three consecutive years as rabi crop. The plant types were described and Kew accession was found to be unique and better adaptive than that of Sutton samples of *N. damascena*. Morphometric (plant height, primary and total branches/plant, capsule/plant, capsule length, seta/capsule, filled seeds/capsule, seed weight/plant as well as capsule and flower sterilities) and meiotic (mean chromosome association/cell at metaphase I, bivalent configurations, chiasma/nucleus, anaphase I segregation and pollen fertility) parameters were assessed in the plant types and statistical analysis (χ^2-test of heterogeneity and Student t-test) of the accumulated data revealed significant variations among/between plant types for most of the traits. Results indicated the possibility of efficient breeding between species/accessions for enhancing gene pool of *Nigella*.

Keywords: Efficient breeding, Meiosis, Morphometric traits, *Nigella damascena*, *Nigella sativa*.

INTRODUCTION

Nigella sative L. (common name – black cumin; Family: Ranunculaceae; annual herb with potential therapeutic uses and spice yielding property of commerce) is reported to be a model plant species for cytological and cytogenetical studies (Datta and Biswas 1985; Datta and Saha 2001; Datta and Rang 2001; Rang and Datta 2001; Saha and Datta 2002; Ghosh and Datta 2006). On the contrary, *N. damascena* L. (common name – 'love-in-a-mist'; Family: Ranunculaceae) is reported from India (Anonymous 1966) and grow in gardens throughout temperate regions of the world (vide Encyclopedia Britannica @ 1999-2000) and is a native of Mediterranean region. Efficient breeding endeavour between the species may widen the gene pool in *Nigella* for selection of desirable 'plant type(s)' of commercial interest. With the view to it, seeds stocks of *N. sativa* and *N. damascena* (two germplasm source used – one from Sutton and Sons', Kolkata, while the other from Kew garden, London) were grown (experimental field plots of Kalyani University, West Bengal plains, Nadia) for three consecutive years and described. Phenotypic variables and meiotic parameters were analysed in the plant types to assess significant variations, if any, for further exploration. The objective of studying meiosis is to understand the process (dealing with the course of microsporogenesis) which is pivotal in designing future programme on reproduction, fertility, genetics and plant breeding.

MATERIAL AND METHOD

Plant types: The seed samples of *Nigella sativa* L. (cultivated variety – *Persian Jewels*, obtained from Sutton and Sons', Kolkata; seed moisture content 7.5%; designated as NS) and *N. damascena* L. (cultivated variety – *Miss Jekyll blue*, obtained from Sutton and Sons', Kolkata, seed moisture content 13.33%, designated as NDS; seed samples obtained from Royal Botanical Garden, Kew, London – accession No. 0016287, designated as NDK) were grown in the Experimental field plots of Department of Botany, Kalyani University (West Bengal plains, Nadia, latitude 22°50′ to 24°11′ N, longitude 88°09′ to 88°48′ E, elevation 48 ft. above sea level, sandy loamy soil) as rabi crop during the months of November to mid-March for *N. sativa* and November to early May for *N. damascena* for three consecutive years. Seeds of the plant types were sown in lines keeping uniform distance between plants (25 cm apart) and lines (30 cm interval). Uniform cultural practices were maintained throughout and no fertilizer application was made

Morphometric analysis: Morphological attributes as presented in Table 1 were studied from 30 plants of *N. sativa*, 27 plants of *N. damascena* – Sutton and 30 plants of *N. damascena* – Kew (in all the plant types 9 to 10 plants were screened in each year randomly, excluding boarder plants; plants were scored from 3 different lines for each plant type for a year). Data recorded were pooled over the plants for each year and over the years for each plant type. Out of 27 NDS plants 17 were seedless. Flower (flowers not transformed to capsules were considered sterile) and capsule (capsules not yielding good filled seeds

were identified as sterile) sterilities, size and colour of the flowers (Horticultural Colour Chart I and II - 1968) and day to first flowering were also analyzed in the plant types.

Meiosis: Meiotic analysis was performed from 2 to 3 randomly selected plants of each plant type in each year. Suitable sized flower buds were fixed in acetic alcohol (1:3 v/v) for 24 hours, transferred to 70% alcohol and stored in a refrigerator for convenient use. Anther squash preparations were performed and PMCs were stained in 1% acetocarmine (Marks 1957). Fully stained pollen grains were considered fertile. Metaphase I (MI) configurations, chiasma per nucleus, anaphase I (AI) segregation and pollen fertility percentage were assessed and data were pooled over the plants and over the years for each plant type. Photomicrographs were taken from temporary squash preparations.

Statistical analysis: χ^2-test of heterogeneity (2 DF) was performed for phenotypic variables and meiotic parameters to assess significant variation among the three plant types under study. Test of significance (Student t-test) was also made for different morphological traits between *N. damascena* – Sutton and Kew samples to estimate significant variation between them.

FIGURE LEGENDS

Figure plate I (1-6) showing plant types, flowers and fruits of *Nigella* **(1)** N. *sativa* (NS); **(2)** *N. damascena* – Sutton (NDS); **(3)** *N. damascena* – Kew (NDK); **(4)** flowers – **(a)** NDK; **(b)** NDS; **(5)** *N. damascena* showing pigmentation on the suture of fruits; **(6)** fruits – **(a)** NS; **(b)** NDS; **(c)** NDK.

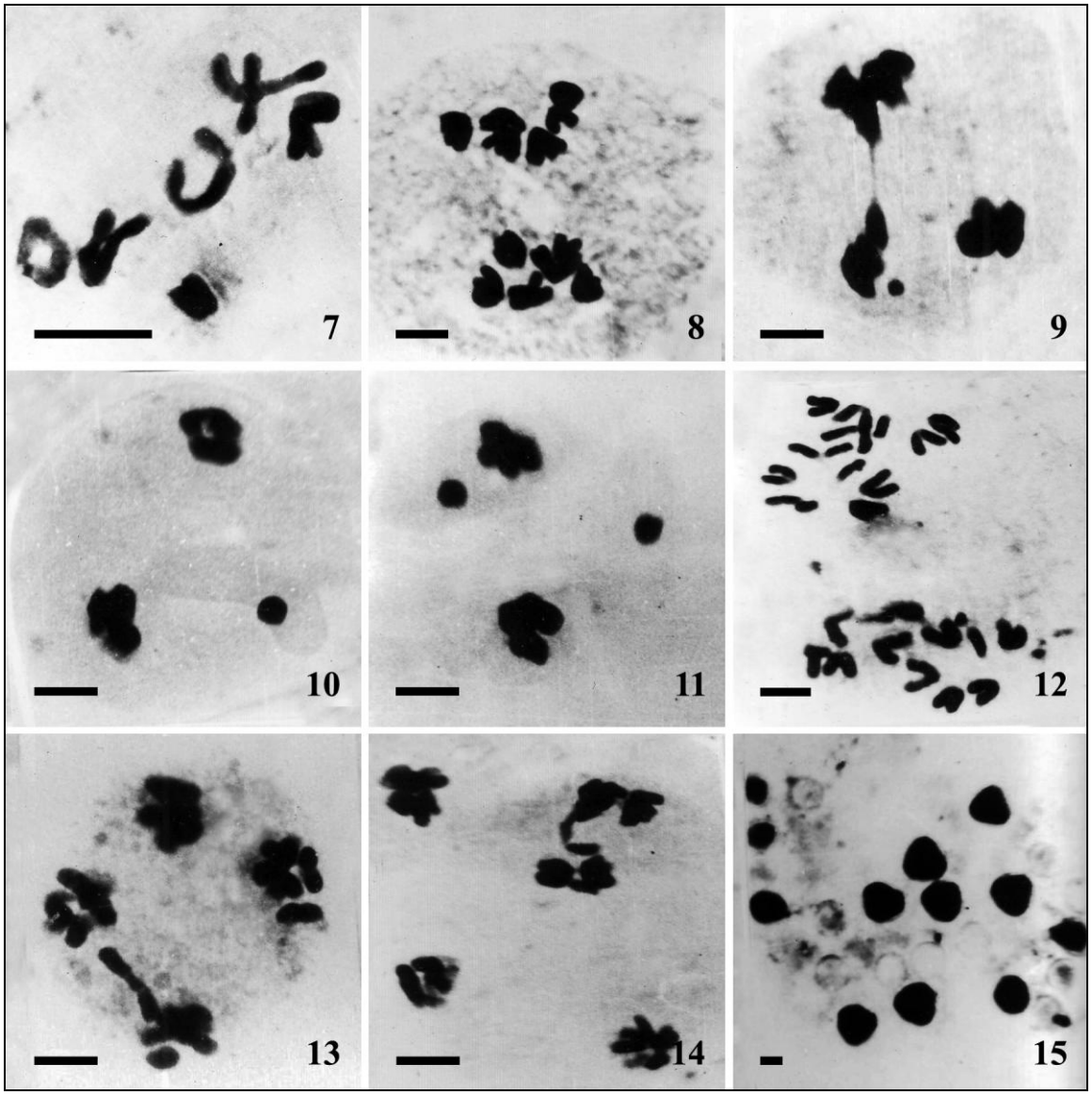

Figure plate II (7-15) showing meiotic configuration and pollen fertility in *Nigella* spp. **(7)** 6II at MI; **(8)** 6/6 separation of chromosomes at AI; **(9)** bridge with a fragment and tripolarity at AI; **(10-11)** AI with laggards; **(12)** irregular separation at MII; **(13-14)** bridge formation at AII; **(15)** stained and fertile pollen grains. Scale bar = 10 µm.

RESULTS AND DISCUSSION

N. satva is an erect (**Fig. 1**) annual herb with pale (French Blue 43/3) flowers (size: 2.74×2.78 cm) without involucres and borne solitary on long, erect peduncles. Flowers were with one whorl of petalloid sepals and they were broad and ovate in shape. Floral shoots were non-pigmented. Capsules were elongated to roundish in shape with no pigmentation (**Fig. 6a**) on the suture and capsules were without involucres of bracts. In NS, the vegetative phase (from germination to early flowering) was noted to be 64 to 80 days; while, the reproductive phase was assessed to be 40 to 55 days. The plants are harvested within 125 to 145 days from sowing. Viability of seeds ranged between 80.0% and 96.0% over the generations. *N. damascena* – Sutton is a bushy, semi-prostrate (**Fig. 2**), annual herb with large number (4 to 11, mean 10.81±0.82) of lax natured pigmented lateral branches; while NDK plants were prostrate at seedling stage but at maturity they were rosette in appearances due to the formation of short sized, compact and non-pigmented lateral branches (8 to 39, mean 22.03±1.12). In NDS, the flowers were large (3.23 cm×3.25 cm), gentian blue (42) with dense, finely cut involucres (**Fig. 4a**), 3 to 5 whorls of petalloid sepals (30 to 40) imparting ornamental value to the plants; sepals were narrow, elongated in nature; capsules were globular-oblong, inflated and with involucres of bract, plum purple (934) pigmentation on the suture. In NDK, the flowers were also large (3.86 cm×3.77 cm), sea blue (0 43/1) with finely cut involucre and with one whorl of petalloid sepals (**Fig. 4b**). Capsules in the plant types

were globular-oblong, inflated and with finely cut involucres and plum purple (934) pigmentation on the suture (**Figs. 5c, 6**). In both the plant types of *N. damascena* vegetative phase (NDS – 145 to 160 days; NDK – 130 to 150 days) was much prolonged than reproductive phase (NDS – 28 to 33 days; NDK – 41 to 60 days) consequently had delayed harvesting (NDS – 185 to 200 days; NDK – 193 to 200 days from sowing) compared to NS.

Morphometric and meiotic parameters studied in the plant types are presented in Table 1. Among the assessed morphological attributes capsule length was found to vary at 5% level, while all other traits (excepting seta/capsule) showed significant variation among the plant types at 0.001 probability level. Test of significance (t-test at 55 DF) conducted between NDS and NDK for the morphological parameters revealed that for excepting plant height ($t=0.37$, $p>0.05$) all other attributes (primary branches/plant: $t=8.07$, $p<0.001$; total branches/plant: $t=5.93$, $p<0.001$; capsule/plant: $t=7.69$, $p<0.001$; capsule length: $t=9.0$, $p<0.001$; seta/capsule: $t=14.45$, $p<0.001$; filled seeds/ capsule: $t=14.45$, $p<0.001$; seed weight/plant: $t=25.5$, $p<0.001$) varied significantly.

All plant types showed 2n=12 chromosomes uniformly in their meiocytes (**Figs. 7, 8**). Mean chromosome association per cell was 5.95II+0.10I in NS, 5.79II+0.41I in NDS and 5.83II+0.33I in NDK respectively. The bivalents formed ring and rod configurations. Frequency of ring bivalent per cell was higher than rod per cell in NS but rod/cell was enhanced than rings in NDK. Although bivalent frequency per cell was consistent ($p>0.05$), univalent/cell ($p<0.001$), chiasma /nucleus ($p<0.001$), ring ($p<0.05$) and rod ($p<0.05$) configurations of bivalents were non-randomly distributed among the plant types. AI segregation of chromosomes was nearly balanced (6/6) in NS (99.49%) and NDK (98.37%) but NDS (6/6 – 77.02%; rest of the cells showed unequal segregation of chromosomes, laggards, tripolarity and bridge formation with or without an accompanying fragment – **Figs. 9, 10, 11**) had disturbed separation to an extent. AII cells studied in the plant types were mostly cytologically balanced rare often irregular separation of chromosomes, laggards and bridges were observed in NDK (**Figs. 12, 13, 14**). Pollen fertility (**Fig. 15**) was recorded to be 98.06% in NS, 88.01% in NDS and 81.61% in NDK. Meiotic data therefore indicated that extremely low amount of seed yield in NDS is rather not cytological, it possibly may have genetic basis.

Table 1. Cytomorphological attributes in *N. sativa* and *N. damascena* plant types

Attributes	NS	NDS	NDK	P value of χ^2-test of heterogeneity at 2 DF
Morphological				
Plant height (cm)	52.18±4.42	42.27±1.56	43.35±1.43	<0.001
Primary branches/plant	7.00±0.71	10.81±0.82	22.03±1.12	<0.001
Total branches/plant	22.50±4.11	27.19±3.63	71.10±5.95	<0.001
No. of capsule/plant	20.00±3.71	9.52±1.92	44.67±3.81	<0.001
Capsule length (cm)	1.03±0.13	1.42±0.03	1.78±0.03	<0.05
Seta/capsule	5.10±0.10	5.30±0.11	5.03±0.01	<0.70
Filled seeds/capsule	59.29±3.20	6.14±1.53	46.59±2.16	<0.001
Seed weight/plant (gm)	1.91±0.38	0.01±0.01	1.03±0.19	<0.001
Flower sterility (%)	11.11	66.07	37.18	<0.001
Capsule sterility (%)	5.50	84.44	47.39	<0.001
Meiosis				
Bivalent/cell	5.95	5.59	5.83	>0.05
Univalent/cell	0.10	0.41	0.33	<0.001
Ring/cell	3.18±0.34	2.98±0.27	2.75±0.18	<0.05
Rod/cell	2.77±0.35	2.86±0.28	3.07±0.19	<0.05
Chiasma/nucleus	9.34±0.28	8.94±0.22	8.35±0.25	<0.001
Total cells scored at MI	175	150	148	-
Equal (6/6) AI separation (%)	99.49	77.02	98.37	-
Total AI cells scored	394	148	184	-
Pollen grains fertility (%)	98.06	88.01	31.61	-
Total pollen grains scored	1174	344	1383	-

CONCLUSION

Results of the present investigation suggested better adaptability of Kew-accession of *N. damascena* in Nadia than Suttons' variety. Cytomorphological variations encountered among the plant types may be explored through efficient breeding and selection. However, necessary approaches must be undertaken to shorten the growth period of *N. damascena* and make it near comparable to *N. sativa* for performing any breeding experiments.

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MYCORRHIZAL INOCULATION EFFECT ON GROWTH RESPONSES AND DRY MASS PRODUCTION OF *MIMOSA HIMALAYANA* GAMBLE SEEDLINGS

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Abstract: *Mimosa himalayana* is a nitrogen fixing shrub of Central Himalayan region. In the present study, effect of mycorrhizal inoculation was observed on the seedlings of *M. himalayana.* For this, seedlings of *M. himalayana* were raised in polyethylene bags containing sterilized mixture of soil and commercial sand. The seedlings of *M. himalayana* became colonized when inoculated with vesicular-arbuscular mycorrhizal fungi. When compared with uninoculated seedlings, inoculated seedlings showed increased root and shoot length with 48% to 58% mycorrhizal dependency for total seedling biomass. Present study suggested that the vesicular arbuscular mycorrhizal fungi act as an important biological factor that contributes to the efficiency of nutrient uptake and use.

Keywords: Colonization, Inoculation, *Mimosa himalayana*, Mycorrhiza, Production

INTRODUCTION

Degradation of land subsequent to deforestation is one of the major ecological problems in the developing world, especially in mountain areas (Echolm 1975). Growing demand for fuel, fodder, wood and food has extensively depleted protective plant cover and exposed soil surface to processes of degradation, resulting in partial to complete loss of soil productivity (National Wasteland Development Board 1987). Mycorrhizal fungi are likely to be most beneficial in diverse (degraded) ecosystems where the production of plants able to form mycorrhiza is high and nutrient deficiencies are an important limitation to plant growth. These fungi can make an important contribution to the rehabilitation of degraded lands, as mycorrhiza can increase the apparent size of the pool of soil nutrients (Barea *et al*.1990). It indicates that mycorrhizal plants can either use available N forms more efficiently, or derive nutrients from sources less available to non-mycorrhizal plants.

The *Mimosa himalayana* Gamble (Family Mimosacae) is a large straggling very prickly shrub with terminal elongate clusters of many tiny pink flowers in globular heads and with twice-pinnate spiny leaves. It is a nitrogen fixing leguminous plant that form root nodules with *Rhizobium* sp. (Singh and Pokhriyal 1997) and grow upto 1600 m asl along the water courses and scrub jungles. Its ability to fix atmospheric nitrogen through biological nitrogen fixation makes it useful in afforestation and reclamation of degraded and nutrient-poor lands. In the present study the influence of VAM fungi on growth of *M. himalayana* seedlings was analyzed. Main objectives of the present experiment were: (i) to determine the potential of *M. himalayana* seedlings to be colonized by VAM fungi; (ii) to quantify the effect of colonization by VAM fungi on seedlings of *M. himalayana.*

MATERIAL AND METHOD

Seedlings of *M. himalayana* were raised from the seeds of the current year crop collected from a forest stand near Nainital town (29°22' N lat. and 79°25' E long.) in the Kumaun region of the Central Himalaya. Seeds were rinsed and surface sterilized (30% H_2O_2 w/v for 20 min). These seeds were sown in polyethylene bags containing 1 kg commercial sand and sieved forest soil in 3:1 ratio (autoclaved twice at 120^{o}C for 1 h, 2 days). VAM inoculation was done with 1 g of surface sterilized infected fine *M. himalayana* root fragments following Tewari *et al* (2003). The inoculum was added to bags while sowing just below the seeds. In addition, uninoculated plants were also maintained as control. For *Rhizobium* inoculation healthy *M. himalayana* nodules were collected from the field. Fresh young nodule lobes were cleansed of soil and homogenized with distilled water in a blender. Inoculum was applied on the surface of soil as liquid suspension when seeds were sown

After establishment one plant per bags was maintained and bags were randomly arranged in block in the glasshouse. Five plants from each sub treatment were uprooted for observation at 6 and 12 months interval. Plant height, root length were recorded. Weight of stems, leaves and roots were estimated by drying them in an oven at 80^{o}C for 24 hour.

AM colonization was observed following root clearing method (Phillips and Haymann 1970). The colonization (%) was calculated by using Nicolson's (1975) formula:

Colonization (%) =

$$\frac{\text{Root segments colonized with VAM}}{\text{Total number of root segments observed}} \times 100$$

The mycorrhizal inoculation effect (MIE) was calculated using the formula given by Bagyaraj (1992):

$$MIE = \frac{\text{Dry weight of inoculated plant-dry weight uniculated plant}}{\text{Dry weight of inoculated plant}} \times 100$$

RESULT

Effect of inoculation on colonization

Colonization developed in all seedlings of *M. himalayana* at each harvest except in the control without inoculation. Hyphae and vesicles were present but no arbuscules were observed. The inoculated seedlings showed 70% colonization by VAM fungi.

Effect of inoculation on seedling growth

Inoculation with VAM fungi significantly increased growth of *M. himalayana* seedlings at each harvest (Table 1). In one-year old seedlings of *M. himalayana*, increment in root and shoot length was 1.9 and 3.1 cm, respectively

The dry mass of *M. himalayana* was significantly greater in plants inoculated with VAM fungi (Table 2). The ANOVA calculated for dry mass yield clearly indicated significant difference in respect to harvest, inoculation and their interaction (Table 3). Mycorrhizal inoculation effect (MIE) was greater on leaf at first harvest and on stem at second harvest (Table 2). In terms of total dry mass MIE increased from Ist harvest (48%) to second harvest (58%). Root: shoot ratio decreased in inoculated plants while leaf weight ratio and stem weight ratio increased.

DISCUSSION

Roots of *M. himalayana* were colonized by VAM fungi in nature and the seedlings of this species were colonized in the glass house experiment when inoculated. Colonization consisted of huphae and vesicles. No arbuscles of VAM fungi were present in the roots of *M. himalayana*. Successful establishment of most woody legumes depends on their ability to form symbiotic association between their roots and beneficial microorganisms like rhizobia and mycorrhizas (Barea et al 1990). Different types of mycorrhizal fungi form associations with plant roots, but the arbuscular mycorrhiza are by far the most widespread type of mycorrhiza in nature and are also the most commonly occurring on nodulated nitrogen fixing plants (Barea *et al*., 1992; Roskoski *et al*., 1986).

VAM fungi have potential to increase growth of host plants (Carey *et al* 1992, Bryla and Koide 1990) thus, inoculation with VAM fungi resulted on an average 93% increase at first harvest and 140% increase at second harvest in total seedling dry mass of *M. himalayana* seedlings. Similar results were reported for other leguminous plants viz. *Gliricidia sepium* (Twum-Ampofo K. *2008*), *Albiziz saman* (Rahman *et al* 2004), *Acacia nilotica* (Sharma *et al* 1996), *Indigofera heterantha* (Bargali, 2006). The presence of mycorrhizal fungi in the rhizosphere greatly affect the nutrient mobilization by producing enzymes and low molecular weight organic acids that interact with soil compound resulting in the increased nutrient availability and uptake for symbiotic plants and improve the growth of the plants. Root: shoot ratio decreased in inoculated plants while Leaf weight ratio and Stem weight ratio increased. Bargali (2006) observed that the mycorrhizal fungi increased the absorption surface of the plants and resulted in increased allocation of biomass to the aboveground parts.

Woody legumes are useful for revegetation of water deficient ecosystems that have low availability of N, P and other nutrients. The scarcity of available phosphorus and the imbalance of trace elements in degraded ecosystems actually limit legume establishment and N_2- fixation. But when associated with mycorrhiza it is reported to increase the establishment of legume (Barea *et al* 1992). In addition, woody legumes exhibit a considerable degree of dependence of mycorrhizae to thrive in stressed situation (Osonubi *et al* 1991). In this study, *M. himalayana* seedlings showed 48 % mycorrhizal dependency at first harvest and 58% at second harvest, respectively. In the Himalayan region some leguminous plants like *M. himalayana* grow on nutrient poor soils such as eroded lands, rocky surfaces etc. Though, these plants themselves do not yield economic goods, but they can improve fertility of soil as well as growth of economically important non nitrogen fixing plants. Thus, VAM associated *M. himalayana* seedling can play a crucial role in restoration of degraded wastelands and can be used to stabilize degraded areas all over Himalaya.

Table 1. Effect of AM inoculation on *M. himalayana* seedlings.

Months after germination	**Parameters**	**Treatments**		**Increment**
		Inoculated	Uninoculated	
6	Root length(cm)	20.3 ± 0. 858	18.5 ± 0.685	1.8
	Shoot length (cm)	15.0± 0.068	10.8 ± 0.244	4.2
12	Root length(cm)	22.1 ± 0.904	20.2 ± 0.668	1.9
	Shoot length (cm)	15.2 ± 0.085	12.1 ± 0.287	3.1

Table 2. Effect of AM inoculation on growth performance of *M.himalayana* seedlings in a glass house experiment (Mean ± SE).

Parameters	**Ist Harvest**			**II nd Harvest**		
	+ VAM	**-VAM**	**MIE(%)**	**+ VAM**	**-VAM**	**MIE(%)**
Leaf dry mass (g/seedling)	0.205±0.0008	0.058±0.0007	71.70	0.451±0.0007	0.152±0.0007	66.29
Stem dry mass (g/seedling)	0.212±0.0007	0.085±0.0006	59.90	0.475±0.0006	0.155±0.0005	67.36
Root dry mass (g/seedling)	0.195±0.0007	0.174±0.0005	10.76	0.380±0.0004	0.236±0.0004	37.89
Total seedling dry mass (g/ seedling)	0.612±0.0008	0.317±0.0036	48.20	1.306±0.0018	0.543±0.0013	58.42
Root: shoot ratio	0.467	1.216	-	0.410	0.769	-
Leaf weight ratio	0.335	0.182	-	0.345	0.279	-
Stem height: Stem dry wight ratio	0.346	0.268	-	0.363	0.285	-
Relative growth rate (g g^{-1} d^{-1})	-	-	-	0.0041	0.0029	-

Table 3. Analysis of variance for total seedling biomass.

Source of variation	Degree of freedom	Sum of square	Mean Square	F value	Significance level
Replicate (R)	4	0.008	0.002	3.03	P< 0.05
Harvest (H)	1	1.05	1.05	1606.06	P< 0.001
Inoculation(I)	1	1.39	1.39	2106.06	P< 0.001
Interaction (H x I)	1	0.283	0.283	428.78	P<0.001
Error	12	0.008	0.00066		
Total	19	2.739	0.144		

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ANALYSIS OF QUALITATIVE TRAITS IN OKRA [*ABELMOSCHUS ESCULENTUS* (L.) MOENCH] GROWN UNDER TWO ENVIRONMENTS

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Abstract: Besides Okra is a potential fibre yielding crop as because the bast fibre is strong, hydroscopic and resistant to rot, thus suitable to meet the global demand as an additional source of ecofriendly fibre. Fifteen genotypes of Okra were evaluated for morphological and yield related traits. Estimation of biochemical constituents i.e. total soluble solids, crude fibre, total cartenoids, calcium and phosphorus were also performed.

Keywords: *Abelmoschus esculentus*, Analysis, Okara

ITRODUCTION

Okra or Bhindi [*Abelmoschus esculentus* (L.) Moench] is an important vegetable crop in the tropics and the subtropics. According to Joshi and Hardas (1976), the cyto taxonomy of *Abelmoschus esculentus L.* (2n = 130) is very complex and confuse that Asian origin of whole or part of the cultigens and does not seem impossible. Indian Okra is quiet different from African Okra, in respect of genomic complements and hybrids between African and Indian varieties show some sterility, the crop to perhaps at turn out be polyphylatic. The cultivated Okra may not probably be a single species but a polytypic complex which exhibits both high polyploidy and hybridity and have which the parental wild species go undetermined.

METHODOLOGY

Its tender fruits contain vitamins A, B, & C – a rich source of iodine and essential micronutrients comprising of calcium, potassium and other mineral matters. The pods are also good source of proteins (1.9%), Carbohydrates (6.4%), fat (0.2%) and fibre (1.2%). The mucilaginous extracts, mostly acetic polysaccharides are commonly employed in India for clarifying sugarcane juice in gur manufacturing.

The experiment was laid out in Randomized Block design having three replications of each genotype in March 2005 (Summer crop) and July 2005 (Rainy season crop). The plot size was kept at 2 mt x 1.5 mt for both the seasons. Spacing of 30 cm x 30 cm for summer crop and 50 cm x 40 cm for Rainy season crop was taken ensuring 24 plants and 20 plants per plot for the respective seasons.

Total Soluble Solids (^{0}Brix)

The value of total soluble solids (TSS) of each sample was determined with the help of a hand refractometer and values were corrected at 20^{0}C (A.O.A.C., 1975).

Crude Fibre (in percent)

The estimation was done according to the method suggested by (A.O.A.C., 1975).

Calcium (g)

The estimation was done according to the method as suggested by (A.O.A.C., 1975).

Total Carotenoids (mg/100 g)

The estimation was done according to the method as suggested by (Mahlberg, P.G. and Venkteswarm, S. (1966).

Phosphorus (mg/100 g)

Total 'P' in plant material was determined by dry – ashing procedure developed by Chapman and Pratt (1961).

ANALYSIS AND DISCUSSION

Analysis of Variance (Table 5) showed that the genotype possessed significant differences for almost all the traits studied under both summer and Rainy seasons. Among the qualitative traits total soluble solids, total carotenoids, crude fibre and phosphorus showed significant differences, while calcium failed to do so (Table 5).

Range

During summer season a wide range of variation was observed The biochemical constituents like total soluble solids (^{0}Brix) (4.4 – 7.3), total carotenoids (mg/100 g) (0.09 – 0.60), (crude fibre (percent) (1.7 – 6.7), calcium (mg/100 g) (0.49 – 0.73) and phosphorus (mg/100 g) (0.42 – 0.53) showed optimum variation among the treatments (Table-1)

Phenotypic and Genotypic Variability

Among the biochemical constituents the genotypic coefficient of variation (GCV) was observed to be highest in total carotenoids (70.15) followed by crude fibre (36.26) under summer environment while during rainy environment crude fibre (28.32) was much higher than the rest of qualitative characters. Similarly the phenotypic coefficient of variation (PCV) Values were high in the same order as in GCV for summer environment while during rainy season crude fibre (42.66) followed by calcium (20.17) was observed**.**

Heritability (in Broad Sense) and Genetic Advance

All the characters under study showed moderate to high heritability coupled with various levels of genetic advance.

Most of the biochemical characters showed low genetic advance (GA)(0.01 – 2.35) in association with high (69.6 – 72.8) to moderate (27.8 – 43.8) and low (6.4) heritability during summer environment. The best combination was observed in crude fibre percent (h^2% 72.8) and GA (2.35). GA as percent of mean was highest in total carotenoids (94.59) followed by crude fibre (63.85).

During rainy season high heritability with high GA was observable in total fresh yield per plant

With respect to biochemical parameters, the highest being in phosphorus (h^2% 44.7) followed by crude fibre percent (h^2% 44.1). All the qualitative traits showed very low level of genetic advance, the highest being in crude fibre (1.37). The GA as percent of mean was highest in crude fibre (38.92).

Phenotypic Correlation

The correlation studies (Table 2, 3 and 4) among the biochemical constituents as well as with total fresh yield per plant did not reveal significant negative and positive associations under both the environments.

Genotypic Correlation

The genotypic Correlation With respect to qualitative Characters (Table 3) total fresh yield per plant was found to have positive correlation with calcium content under both the environments. Negative and significant association between characters viz. Calcium content and total soluble solids, calcium content and total carotenoids and crude fibre were observed during summer environment. Similarly under rainy environment phosphorus was found to have negative and significant association with calcium. Similar trend was observable with respect to total carotenoids content and crude fibre.

Environmental Correlation

The present investigation was carried out under two environments which aptly recognizes the importance of environmental correlations with respect to two distinct climatic situations.

None of the biochemical constituents were found to have any sort of significant correlation under both the environments.

To achieve the above objectives, field experiments were conducted at the Agricultural Experimental farm of Calcutta University at Baruipur South 24-Parganas during summer and rainy season of 2005. Estimation of biochemical constituents were simultaneously conducted in the laboratory of Department of Horticulture, Institute of Agricultural Science, University of

Calcutta. The meteorological data pertaining to the period of experimentation have been depicted in (Table – 6). The recorded data of average monthly maximum and minimum temperature, relative humidity and total rainfall at the experimental station during the course of investigation are presented.

Among the biochemical constituent's high to moderate range of heritability in association with low GA was observed for characters like, crude fibre, total soluble solids, total carotenoids and phosphorus. While, low heritability in association with GA was seen in calcium. In characters which were having high heritability with high genetic advance indicates that such characters are controlled by additive action of the polygenes and are more reliable for selection. Average heritability with average to low genetic advance suggested that such characters under the influence of non-additive.

Table 1. Mean and Estimation of Genetic Parameters for Qualitative Traits in Okra Grown under Two Environments.

Characters	**Env**	**Grand Mean**	**Range**	**G.C.V.**	**P.C.V.**	**(h^2 %)**	**G.A.**	**GA as % of Mean**
Total soluble solids (^{0}Brix)	S	6.29	4.4 – 7.3	10.93	13.10	69.6	1.18	18.75
	R	6.20	4.7 – 7.1	8.36	12.95	41.7	0.69	11.12
Total Carotinoids (mg/g.)	S	0.14	0.09 – 0.60	70.15	105.96	43.8	0.14	94.59
	R	0.11	0.10 – 0.15	11.95	19.00	39.6	0.02	17.24
Crude Fibre (%)	S	3.68	1.7 – 6.7	36.26	42.51	72.8	2.35	63.85
	R	3.52	1.8 – 6.5	28.32	42.66	44.1	1.37	38.92
Calcium (mg/100g)	S	0.55	0.49 – 0.73	4.87	19.17	06.4	0.01	1.81
	R	0.54	0.44 – 0.66	8.49	20.17	17.7	0.04	7.40
Phosphorus (mg/100 g)	S	0.47	0.42 – 0.53	6.64	12.60	27.8	0.03	6.38
	R	0.47	0.42 – 0.57	7.34	10.98	44.7	0.05	10.63

Note : S – Summer
R – Rainy.

Table 2. Phenotypic correlations among biochemical constituents and total fresh yield per plant in Okra grown under two environments.

TRAITS	Total soluble Solids	Total caroti-noids	Crude Fibre	Calcium	Phosp-horus	Total Fresh yield/ Plant
Total soluble solids		0.181	-0.231	-0.186	0.106	-0.186
Total Carotinoids	-0.096		-0.212	-0.008	0.063	0.197
Crude Fibre	-0.144	-0.112		0.048	-0.186	-0.149
Calcium	-0.179	0.017	0.004		-0.221	0.043
Phosphorus	0.144	-0.135	0.070	-0.223		-0.112
Fresh Yield per Plant	0.142	0.177	-0.291	0.100	-0.046	

Note : 'Normal' represents Summer and **'Bold'** represents Rainy.

Table 3. Genotypic correlations among qualitative traits and total fresh yield per plant in Okra grown under two environments.

TRAITS	Total soluble Solids	Total caroti-noids	Crude Fibre	Calcium	Phosp-horus	Total Fresh yield/ Plant
Total soluble solids		0.301	-0.336	-1.026**	0.476	-0.349
Total Carotenoids	-2.258		-0.538*	-1.292**	0.287	0.121
Crude Fibre	-0.297	-0.697**		0.226	-0.216	-0.407
Calcium	-0.294	0.391	-0.223		-0.313	1.225**
Phosphorus	0.209	-0.299	0.247	-0.766**		-0.254
Fresh yield per plant	0.066	0.205	-0.502	0.954**	-0.419	

Note: 'Normal' represents Summer and **'Bold'** represents Rainy.
* and ** are significant at 5% and 1% levels of significance respectively.

Table 4. Environmental correlations among biochemical constituents and total fresh yield per plant in Okra grown under two environments.

TRAITS	Total soluble Solids	Total caroti-noids	Crude Fibre	Calcium	Phosp-horus	Total Fresh yield/ Plant
Total soluble solids		0.037	0.027	0.059	-0.220	0.058
Total Carotinoids	0.014		0.235	0.289	-0.058	0.267
Crude Fibre	-0.029	0.309		-0.002	-0.200	0.275
Calcium	-0.143	-0.123	0.097		-0.218	-0.266
Phosphorus	0.096	-0.017	-0.072	-0.011		-0.027
Fresh Yield per Plant	0.207	0.156	-0.102	-0.289	0.292	

Note: 'Normal' represents Summer and **'Bold'** represents Rainy.

Table 5. Analysis of Variance for different biochemical constituents in Okra grown under distinct environments

Source	D.F.	Env.	Total soluble Solids (^{0}Brix)	Total caroti-noids (mg/g)	Crude Fibre (%)	Calcium (mg / 100 g)	Phosp-horus (mg / 100 g)
Repli-Cation	2	S R	1.32** 0.93	0.0143 0.0002	0.288 1.972	0.004 0.008	0.002 0.001
Treat-Ments	14	S R	1.62** 1.18**	0.046** 0.0083**	6.024** 4.269**	0.012 0.016	0.002 0.005**
Error	28	S R	0.20 0.37	0.013 0.002	0.668 1.26	0.010 0.010	0.002 0.001

Note: * and ** are significant at 5% and 1% levels of significance respectively.
S – Summer
R – Rainy.

Table 6. Meteorological Data of Agricultural Experimental Station, Baruipur, 24 – Parganas (South), West Bengal.

Month of 2005	Total Rainfall (mm)	Temperature (^{0}C)		Relative Humidity (%)	
		Maximum	Minimum	Maximum	Minimum
March	90.2	30.1	20.3	92	43
April	44.6	34.4	24.4	93	57
May	95.0	35.3	25.5	89	59
June	249.4	35.7	27.0	89	65
July	651.7	30.8	24.1	94	68
August	277.2	32.0	26.3	94	77
September	235.9	32.4	26.2	94	76
October	455.2	30.2	24.1	96	75

Source: Dept. of Agro. Meteorology, Writers Building, Kolkata.

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ADOPTION OF ECO-FRIENDLY MANAGEMENT PRACTICES BY VEGETABLE GROWERS

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Abstract: The investigation was undertaken during the year 2008-09 in purposively selected Indore block of Indore district of Madhya Pradesh in terms of socio-personal, economic and communication profile of vegetable growers. Regarding the knowledge about eco-friendly management practices most of the respondents possessed medium to high level of knowledge. Higher percentage of the respondents (61.25%) had medium adoption of eco-friendly management practices.. About 88.22 per cent variation in level of knowledge was contributed by all eleven antecedent variables related to socio-personal, economic and communication characteristics of vegetable growers. Education, mass media exposure, extension participation and information seeking behaviour were positively and significantly influenced the knowledge to the extent of 87.05 per cent. Education showed its superiority over remaining variables in respect of influencing knowledge level. Education had recorded highest percentile contribution (28.93) followed by mass media exposure (24.80), information seeking behaviour (21.50) and extension participation (13.83). About 83 per cent variation in extent of adoption was explained by all eleven antecedent variables. Education, mass media exposure, information seeking behaviour and land holding significantly influenced the adoption of eco-friendly management practices by the vegetable growers to the extent of 80.83 per cent. Education recorded highest percentile contribution (54.70) followed by mass media exposure (34.98) and information seeking behaviour (21.59). Extent of adoption was negatively and significantly influenced by size of land holding to the extent of –2.53 per cent in terms of percentile contribution towards multiple R^2 value.

Keywords: Adoption, Management practices, Vegetable

INTRODUCTION

Vegetables are grown in India since thousands of years but now a day it has become an important enterprise at national and international level. In recent years, the vegetable now become an essential requirement of the daily human diet, because of its nutritional value. In M.P. total area under vegetable cultivation is 663.9 lakh hectares, (2004-2005) with a production of 31.84 lakh tonne and in Indore district 22.68 thousand hectares in 1999-2000 which increased to 26.46 thousand hectares in 2003-04 and later reduced to 22.25 thousand hectares in 2004-05 and further increased to 26.48 thousand hectares in 2006-07 (Source- Commissioner, Land Record, M.P.). The production of vegetable crops in Indore district was at 6352.50 thousand tonnes in 2006-07 and further increased to 6423.90 thousand tonnes in 2007-08 (Source- Government Department of Horticulture, Indore).

The modern agriculture has been successful in meeting the increased food needs of alarmingly growing population. But, the problem associated with modern agriculture like, the high cost of inorganic chemical fertilizers and plant protection chemicals, stagnated yield levels over the years and the mounting health and environmental hazards have forced many farmers and scientists to focus attention on ecologically sound, viable and sustainable alternative non-chemical farming.

MATERIAL AND METHOD

The study was conducted during 2008-09 in Indore district of Madhya Pradesh. The research design adopted for this study was ex-post facto technique. . From Indore district, Indore Block was purposively selected based on maximum area under vegetable crop. Accessibility, time available with the researcher etc. were the other criteria for the selection of this block. There are 16 RAEOs circles in Indore block. Out of 16, 5 RAEOs circles were selected randomly. Two villages from each selected RAEO circle were selected randomly. Their selection was made from the list of villages prepared for each selected RAEOs circle through simple random sampling method. A list of vegetable growers was prepared separately for each of the selected village. From the prepared list, eight vegetable growers were selected randomly for each village irrespective of total number of farmers in that village. Thus, the sample size comprised of 80 vegetable growers. All the respondents were individually interviewed using pre-tested interview schedule. Adoption and knowledge of eco-friendly management practices by vegetable growers are the dependent variables. Based on the scores, the respondents were classified in to the three adopters and knowledge categories viz., low, medium and high using mean and standard deviation as measure of check.

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RESULT AND DISCUSSION

Knowledge of vegetable growers about eco-friendly management practices.

As regard to the knowledge of eco-friendly management practices, majority (56.25%) of the respondents had medium, 26.25 per cent high and 17.50 per cent had low level of knowledge about eco-friendly management practices. The result expressed by the respondents regarding knowledge about eco-friendly management practices was medium.

The findings were in conformity with the findings of Borkar et al. (2000) and Kalashkar et al. (2001),

Table 1. Distribution of vegetable growers according to their overall knowledge level about eco-friendly management practices

Knowledge category	Vegetable growers	
	Frequency	Per cent
Low (< 10.47)	14	17.50
Medium (10.47 – 18.21)	45	56.25
High (> 18.21)	21	26.25

Mean = 14.34 SD = 3.87

Distribution of vegetable growers according to their overall extent of adoption of eco-friendly management practices

It was observed from the data presented in Table 4.7 that majority (61.25%) of the respondents belonged to medium adoption category, whereas, 23.75 and 15.00 per cent of them in high and low adoption categories of eco-friendly management practices, respectively.

This, findings were in accordance with Sriram and Paliniswamy (1999), Chothe and Borker (2000), and Nagdev and Venkatramaiah (2006).

Table 2. Distribution of vegetable growers according to their adoption level of eco-friendly technologies

Adoption category	Vegetable growers	
	Frequency	Per cent
Low (< 24.22)	12	15.00
Medium (24.22 – 31.48)	49	61.25
High (> 31.48)	19	23.75

Mean = 27.85 SD = 3.63

Relationship between personal, socio-economic and communication characteristics of vegetable growers with knowledge and adoption of eco-friendly technologies:

The antecedent variables *viz.* education, size of family, annual income, land holding, occupation, social participation, socio-economic status, mass media exposure, extension participation and information seeking behaviour were found to have positive and significant correlation with the level of knowledge possessed by the vegetable growers regarding eco-friendly management practices.

The antecedent variables studied, education, size of family, annual income, occupation, social participation, socio-economic status, mass media exposure, extension participation and information seeking behaviour were significantly correlated with adoption of eco-friendly management practices by the vegetable growers whereas land holding was found to be non significant. Thus it could be inferred that vegetable growers with higher education, big size of family, higher annual income, subsidiary occupation along with farming, higher social participation, higher socio-economic status, high mass media use and higher information seeking behaviour had high extent of adoption of eco-friendly management practices in vegetable crop.

Table 3. Coefficient of correlation between the consequent variables extent of knowledge and adoption and rest eleven antecedent variables

S. No.	Variables	Knowledge	Adoption
1.	Age	0.175	0.135
2.	Education	0.871**	0.859**
3.	Size of family	0.287**	0.301**
4.	Annual income	0.363**	0.275*
5.	Land holding	0.238*	0.148
6.	Occupation	0.555**	0.416**

7.	Social participation	0.397**	0.353**
8.	Socio-economic status	0.334**	0.221*
9.	Mass media exposure	0.858**	0.811**
10.	Extension participation	0.791**	0.734**
11.	Information seeking behaviour	0.766**	0.760**

* = Significant at p=0.05 ** = Significant at p=0.01

Regression analysis

Level of knowledge

The step down regression analysis depicted that following factors, education, mass media exposure, extension participation and information seeking behaviour had been retained at the seventh step. These, however, elucidated their stupendous contribution (multiple R2=87.05 per cent) towards the total variation 88.22 per cent .It can be stated that only 1.17 percent variation in level of knowledge were unexplained by these four above-mentioned antecedent variables.

Table 4. Step down regression analysis: The 7th step showing regression coefficient of consequent variable level of knowledge on the antecedent variables

S.No.	Variables	BETA	BETA ×R	REG. COEF.-B
1.	Education (X2)	0.265	26.23	0.184*
2.	Mass media exposure (X9)	0.325	31.85	0.301**
3.	Extension participation (X10)	0.185	16.75	0.168*
4.	Information seeking behaviour (X11)	0.288	25.17	0.328**
Multiple R2 = 0.8705				

BETA = Partial contribution towards Y1

BETA×R = Percentile contribution towards R2 value of different antecedent variables

REG. COEF.-B = Regression coefficient of Y on Xi (i = 2, 9, 10, 11)

* = Significant at p=0.05 ** = Significant at p=0.01

Level of Adoption

The multiple R2 being 80.83 per cent, the inferences could be drawn that these four variables viz., education, land holding, mass media exposure and information seeking behaviour out of eleven studied variables had explained a substantive amount of total variation explained by all 11 variables

Table 5. Step down regression analysis: The 7th step showing regression coefficient of consequent variable extent of adoption on the antecedent variables

S. No.	Variables	BETA	BETA ×R	REG. COEF.-B
1.	Education (X2)	0.439	46.83	0.312**
2.	Land holding (X5)	-0.138	-2.54	-0.138*
3.	Mass media exposure (X9)	0.310	31.10	0.302*
4.	Information seeking behaviour (X11)	0.262	24.61	0.314*
Multiple R2 = 0.8083				

BETA = Partial contribution towards Y2,

BETA×R = Percentile contribution towards R2 value of different antecedent variables

REG. COEF.-B = Regression coefficient of Y2 on Xi (i = 2, 5, 9 and 11)

* = Significant at p=0.05 ** = Significant at p=0.01

CONCLUSION

It was concluded regarding the knowledge eco-friendly management practices most of the respondents possessed medium to high level of knowledge. Higher percentage of the respondents (61.25%) had medium adoption of eco-friendly management practices. Ten out of eleven variables, namely education, size of family, annual income, land holding, occupation, social participation, socio-economic status, mass-media exposure, extension participation and information seeking behaviour exhibited significant to highly significant positive association with knowledge of vegetable growers about eco-friendly management practices. All the studied socio-personal, economic and communication variables except age and land holding exhibited positive and significant association with adoption of eco-friendly management practices by vegetable growers.About 88.22 per cent variation in level of knowledge was contributed by all eleven antecedent variables related to socio-personal, economic and communication characteristics of vegetable growers. Education, mass media exposure, extension participation and information seeking behaviour were positively and significantly

influenced the knowledge to the extent of 87.05 per cent.

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SOME STUDIES ON PHYSICAL AND CHEMICAL PROPERTIES OF TAMARIND AT DIFFERENT MOISTURE CONTENT

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Abstract: Tamarind (*Tamarindus indica* L) is an economically important fruit of India as well as Chhattisgarh. The knowledge about physical and chemical properties like size, weight,moisture content, protein content, carbohydrate content *etc.* of any biomaterial is essential to designing its equipment for processing , storage , transportation and for the value addition. In the present investigation, some studies on physical and chemical properties of tamarind at different moisture content were carried out. For the experiment physical and chemical properties were determined at three different moisture- Initial 22.0%(wb), After sun drying 17.90%(wb), After hot air drying 15.80% (wb). physical properties of Tamarind fruit like size, length, breadth, thickness and weight of fruit (pulp weight, seed weight, shell weight etc) followed a declining trend with decrease in moisture content of the tamarind fruit. The chemical properties like total soluble solids, protein content, carbohydrate content, fat and ash content followed an increasing trend but the titratable acidity is decrease with decreasing the moisture content of the fruits and the color of tamarind pulp was clearly observed that it became darker, redder and yellowier than the initial and the total color (ΔE) difference at different treatments is 0, 5.807 and 6.458 under normal, sun dried and hot air dried condition respectively.

Keywords: Tamarind, Physical & chemical properties

INTRODUCTION

Tamarind *(Tamarindus indica L.)* fruit was at first thought to be produced by an Indian palm, as the name tamarind comes from a Persian word 'Tamar-u1-hind', meaning 'Date of India', Its name 'amlika' in Sanskrit indicates its ancient presence in the country. In India, tamarind is known by a wide variety of vernacular names: Assamese – Tetuli; Bengali – amli, nuli, textili tentul; Gujrati – amali, ambali; Hindi – ambli, amli, imli; Kannada – hunase, hunase-mara, hunse; Malayalam – puli; Marathi – amli,chinch; Oriya – koya, tentuli; Punjabi – imli; Parsian – Tamarhindi; Tamil – Puli,pulia-maram; Telugu – Chinta; Urdu – imli. In Arabic it is Tamre-Lindi, in French –tamarind, in Spanish and Portuguese – tamarindo and English-speaking people call it tamarind (Mishra, 1997).

Tamarind is an economically important tree of India as well as Chhattisgarh. In India, it is abundantly grown in Madhya Pradesh, Bihar, Andhra Pradesh, Tamil Nadu and Karnataka (Anon, 1993).

Tamarind is a long living (80-120) years, large spreading tree, upto 30 m high. Generally, the tree begins to bear fruit at the age of 8-12 years and continuous to yield for more than 60 years (Duke, 1981). Tamarind is a highly cross pollinated crop hence a wide variety is common in this spice (Geetha, 1995). Every part of the tree can be put to use. The product of tamarind, leaves, fruits, seeds, flowers, roots have been extensively used in the traditional medicines in India and Africa and several medicinal properties are claimed for the various preparations of tamarind (Ravindran *et al*, 2002).

On an average tamarind pod composed of shell (15-25%), pulp (45-55%), seeds (25-35%), fiber (10-15%). The edible portion of dried tamarind contain moisture (15-30%), protein (2.0-8.79%), tartaric acid (8.0-18.0%), carbohydrates (56.70-70.70%), fibre (2.20-18-30%), reducing sugar (25.0-45.0%), and protein (2.0-4.0%) (Shankaracharya, 1998).

MATERIAL AND METHOD

The work was carried out in the Department of Agricultural Processing and Food Engineering, Faculty of Agril. Engg. and Department of Dairy Chemistry, C.O.D.T. Indira Gandhi Krishi Viswavidyalaya, Raipur (CG).In order to analysis the physical and chemical parameters of tamarind at different moisture content tamarind fruits were kept under three different treatments (before sun drying, after sun drying and after hot air drying up to 100˚C in Tray dryer) and the tamarind at the three moisture content was analyzed for physical and chemical properties of tamarind. For the analysis of physical properties 20 pod were taken randomly and for each parameter measured separately. The different physical properties of tamarind such as size, length, breadth, thickness and weight of fruit were determined using standard techniques. The moisture content of tamarind fruit was determined as per the Standards of American Society of Agricultural Engineers (Anon, 1992). The chemical properties of tamarind such as titratable acidity of tamarind pulp was determined as per the procedure of Ranganna (1986), total soluble solids were determined by using portable hand refractometer, protein of the tamarind pulp was determined by Kjeldahl method (Jackson, 1958), fat content of tamarind was determined by Soxhelet extraction apparatus using petroleum ether of 40-60 °C. Ranganna (1986), ash content of the raw materials was estimated by the dry ashing method as described in the A.O.A.C.(1995), total carbohydrates were calculated by the "By – difference" method as described in the A.O.A.C. (1995), and for the

analysis of color of tamarind pulp the Hunter Lab Color Flex spectrophotometer was used.

RESULT AND DISCUSSION

Physical properties of tamarind fruit

By studying physical properties of Tamrind fruit at different moisture content (table-1,2) the higher fruit length (11.2 cm) was observed at before sun drying, after sun drying (11.2 cm) & after hot air drying (11.1 cm). The higher fruit breadth (2.00 cm) was observed at before sun drying, after sun drying (2.00 cm) & after hot air drying (1.9 cm). The higher fruit thickness (1.2 cm) was observed at before sun drying, after sun drying (1.1 cm) & after hot air drying (1.00 cm). The higher size of fruit (2.99 cm^3 **)** was observed at before sun drying, after sun drying (2.90 cm^3) & after hot air drying (2.71 cm^3). The higher weight of fruit (18.15 g**)** was observed at before sun drying, after sun drying (17.35 g) & after hot air drying (17.10 g). The higher weight of shell (2.15 g**)** was observed at before sun drying, after sun drying (2.05 g) & after hot air drying (2.00 g). The higher weight of shell (0.45 g**)** was observed at before sun drying, after sun drying (0.44 g) & after hot air drying (0.43 g). The higher weight of pulp (9.65 g**)** was observed at before sun drying, after sun drying (8.96 g) & after hot air drying (8.79 g). The higher weight of seed (5.9 g**)** was observed at before sun drying, after sun drying (5.9 g) & after hot air drying (5.88 g).

Table 1. Length, breadth, thickness and size of tamarind fruit at different moisture contents.

Different moisture contents	Physical parameters			
	Length (cm)	Breadth (cm)	Thickness (cm)	Size (cm^3)
Initial (22.0%)	11.20	2.00	1.20	2.99
After sun drying (17.90%)	11.20	2.00	1.10	2.90
After hot air drying (15.80%)	11.10	1.90	1.00	2.71

Table 2. Fruit weight, shell weight, pulp weight, fiber weight, & seed weight at different moisture contents.

Different moisture contents	Physical parameters				
	Fruit wt. (g)	Shell wt. (g)	Pulp wt. (g)	Fiber wt. (g)	Seed wt. (g)
Initial (22.0%)	18.15	2.15	9.65	0.45	5.9
After sun drying (17.90%)	17.35	2.05	8.96	0.44	5.9
After hot air drying (15.80%)	17.1	2	8.79	0.43	5.88

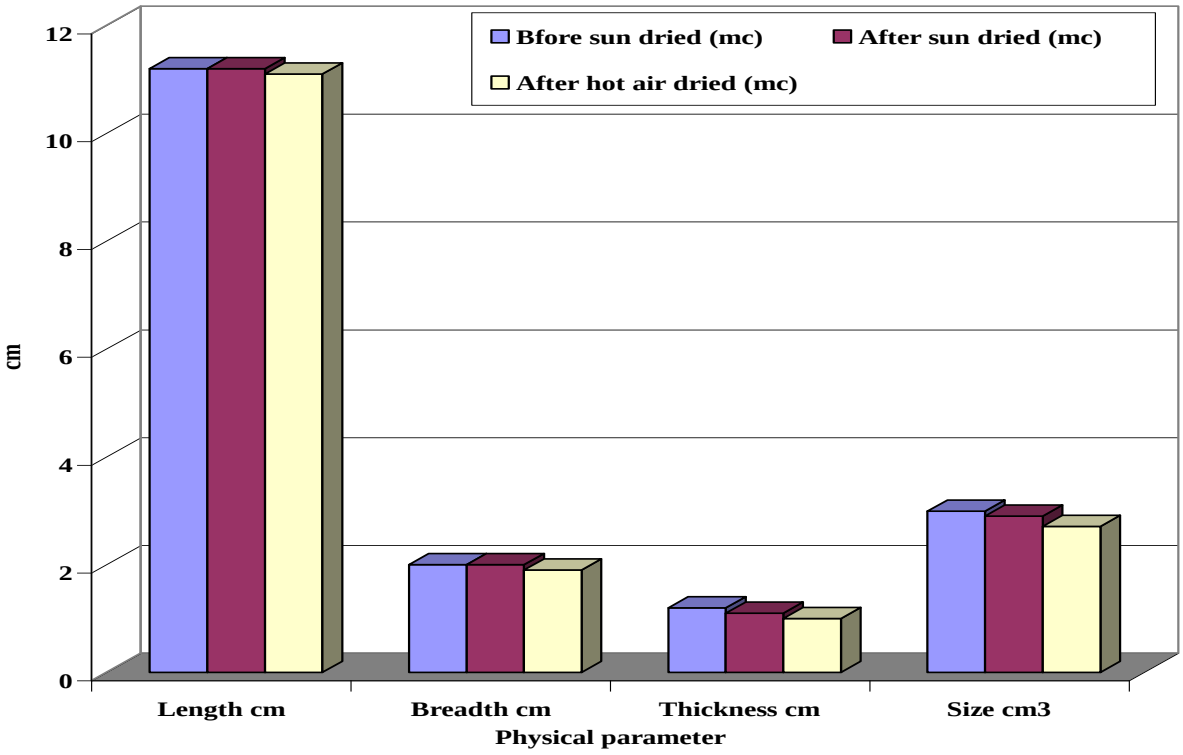

Fig.4.1 Effect of moisture content of fruits in its physical character

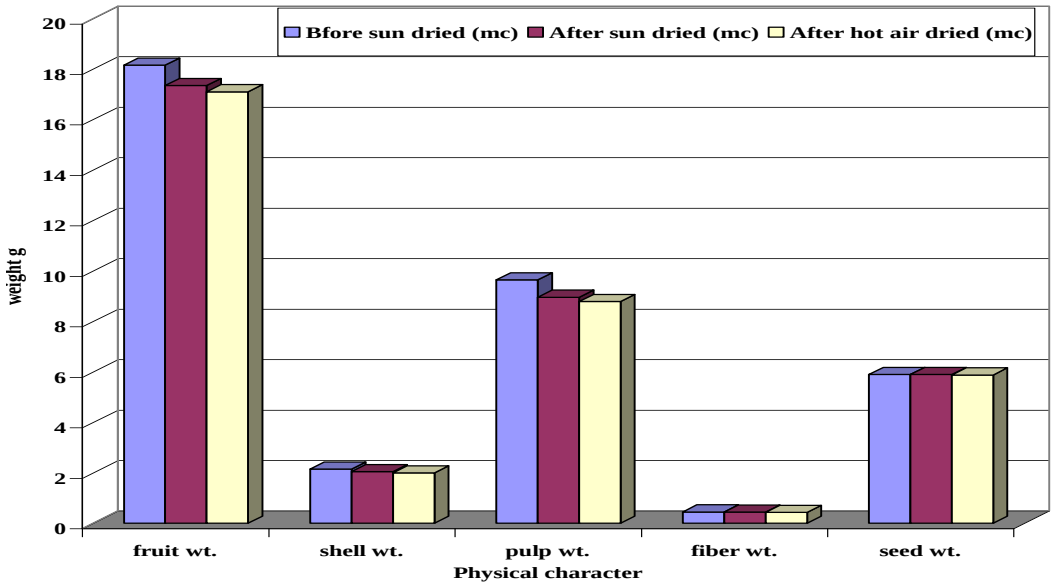

Fig. 4.2 Effect of moisture content of fruits in weight of fruits

Chemical properties of tamarind fruit

By studying chemical properties of Tamrind fruit at different moisture content (table-3) the higher percent of titratable acidity was found in before sun drying of fruit (8.1 %), after sun drying (7.8 %) & after hot air drying (7.5 %). The higher percent of TSS of pulp was found in after hot air drying of fruit (15.79 ° Brix), after sun drying (13.81 ° Brix) & before sun drying (12.33 ° Brix). higher percent of protein content was found in after hot air drying of fruit (3.8 %), after sun drying (3.4 %) & before sun drying (2.85 %). Higher percent of fat content of pulp was found in after hot air drying of fruit (3.8 %), after sun drying (3.4 %) & before sun drying (2.85 %). The higher percent of ash content of pulp was found in after hot air drying of fruit (2.69 %), after sun drying (2.32 %) & before sun drying (1.96 %). The higher percent of carbohydrate content was found in after hot air drying of fruit (77.45 %), after sun drying (75.93 %) & before sun drying (72.78 %). The total color (ΔE) difference at different treatments is 0, 5.807 and 6.458 under normal, sun dried and hot air dried condition respectively.

Table 3. Effect of different moisture content on chemical parameters of tamarind

Different moisture contents	Chemical parameters					
	TA	TSS	Fat	Protein	Carbohydrate	Ash
Before sun drying (22.0 %)	8.10	12.33	0.37	2.85	72.78	1.96
After sun dried (17.90 %)	7.80	13.81	0.45	3.40	75.93	2.32
After hot air dried (15.80 %)	7.50	15.79	0.49	3.80	77.45	2.69

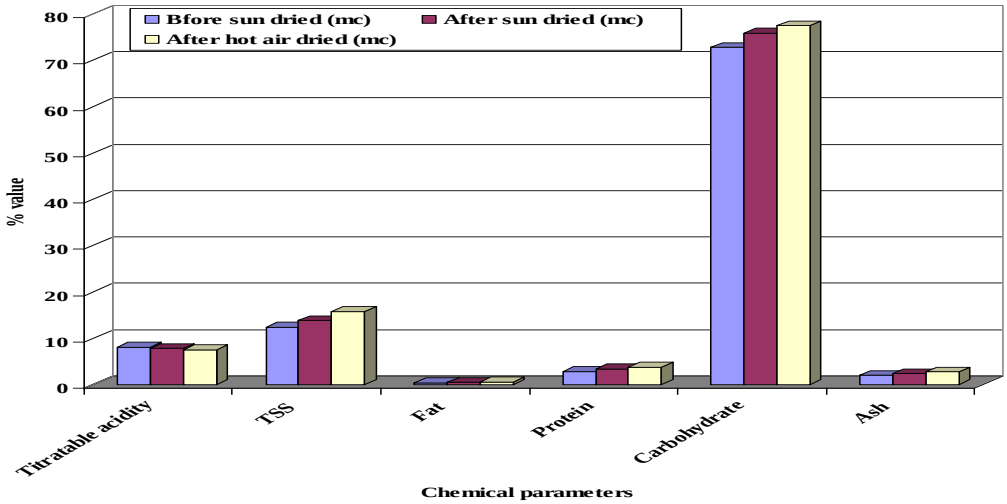

Fig 4.3 Effect of different moisture content of fruits on chemical parameters

CONCLUSION

In conclusion it can be state that the physical properties of Tamarind fruit like size, length, breadth, thickness and weight of fruit (pulp weight, seed weight, shell weight etc) followed a declining trend with decrease in moisture content of the tamarind fruit. The chemical properties like total soluble solids, protein content, carbohydrate content, fat and ash content followed an increasing trend but the titratable acidity is decrease with decreasing the moisture content of the fruits and the color of tamarind pulp was clearly observed that it became darker, redder and yellowier than the initial and the total color (ΔE) difference at different treatments is 0, 5.807 and 6.458 under normal, sun dried and hot air dried condition respectively.

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DISTRIBUTION OF TRACE METALS IN DRINKING WATER OF SOME RURAL HABITATIONS IN WESTERN UTTAR PRADESH, INDIA AND THEIR SUITABILITY FOR DRINKING PURPOSE

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Abstract: A study was conducted to assess the distribution of manganese, copper zinc and iron in drinking water in some part of western Uttar Pradesh. Ground water in the study area is neutral to moderately in nature. It was observed that the ground water in the study area is having higher concentration of iron and zinc which is vulnerable to drink. Iron was much higher than the acceptable limit in approximately 59% of water sample as per guide line of (WHO) However, the concentration of zinc were permissible limit but it was much higher than acceptable limit as per EPA guideline. The concentration of copper and manganese was within the limit. The suitability of ground water for drinking purpose were examined using WHO and EPA classification, which indicate that ground water, was unsuitable for drinking purpose in few location.

Keywords: Trace metals, Drinking water, Manganese, Copper, Zinc, Iron

INTRODUCTION

Ground water is a main source of drinking water in developing world (Datta, P.S. 2005). In rural and small communities, ground water is only source of drinking water (Cantere , 1987). About 95% of rural population living in India depends upon ground water for domestic use (Mohair et al, 2002). Water pollution is a serious problem in India as almost 70% of its surface water and growing number of its ground water resources are already contaminated by biological, organic and inorganic pollutants (Sangu and Sharma, 1987 and Rao and Mamtha 2004). Recently it has been reported that more than 33% country ground water resources are unfit for consumption (Times of India, 2010). The main source of ground water contamination is animal waste, industrial effluents, fertilizer, herbicide, insecticide and fungicide. When these applied to crop land and mix in soil, same residue remains in the soil, after plant uptake and may leach in the ground water. Pollution of water is increasing steadily due to rapid population growth, industrial proliferations, urbanizations, increasing living standard and water spheres of human activities. (Ramkrishnan, C.K. *et al*., 2009 and Nageswera Rao *et al*., 2007). Modern agriculture practices (fertilizer and pesticide application in the field) have adversely effected the environment *e.g.*, ground water contamination with heavy metal. Due to economic region, fertilizers are usually not sufficiently purified during the process of manufacturing, and it contained several impurities, among them heavy metals (Table-1). Fertilizer like super phosphate contains the highest concentration of Cd, Co, Cu, and Zn as impurities. Copper sulphate and iron sulphate have the highest content of Pb and Ni (Eugnia *et al.*, 1995).

The behavior of trace metals in ground water is complicated and occurs due to biogeochemical process (WHO, 1993). A number of trace elements are important for the growth and development in the body for example, Zn is essential dietary element and its deficiency causes hypogonadism (Whitten et al, 2004). Trace metals, such as Cu and Zn are necessary in low concentration for all living organism while excess concentration of these elements is harmfull (Merian, 1991).

MATERIAL AND METHOD

Study area

For the study of the problem different villages around the kali east river in Meerut district were selected. Meerut is situated on the Delhi- Dehradun highway and geographically it is located at 29^0 04' N latitude, 77^0 42' E longitude and at an altitude of 237 meter above the mean sea level. The climate of this region is subtropical characterized with extreme summer as well as winter. The maximum temperature between 43 to 45 ^{0}C is common during summer while low temperature of 3 ^{0}C accompanied by frost may be experienced in December to January. The winters are cool; frost generally occurs at the end of December and may continue till the end of January. There is a long variation in rainfall distribution in this region about 80-90 % of rain fall is received during July to September.

Water sample analysis

The samples were collected after the extraction of water either from privately owned manually operated hand-pumps or from electricity operated bore-wells. The water was left to run from the sampling source for 4–6 min to pump out the volume of water standing in the casing before taking the final sample. Then samples were collected in pre-cleaned, sterilized polyethylene bottles of l litre capacity. The samples were taken by holding the bottle at the

bottom to avoid any contamination and were analyzed just after the sampling. All the samples were stored in a portable Ice box and transported to the laboratory within 5 hour and stored at low temperature. Ground water samplings were analyzed cationic composition in ground waste. The concentration of manganese, copper, zinc and iron was determined by atomic absorption spectrometer (Parkin-Elmer Made 3110). Analytical grade (AR) chemicals and double glass distilled water were used to prepare the reagents. The analysis of various anionic composition were performed according to APHA, 1998 and APHA–AWWA–WPCF(1994).

Table 1. Metal ion accumulation in underground water in left and right hand side villages of near Kali east river.

S.No.	Name of Villages	Manganese	Copper	Zinc	Iron
1.	Satguru Nagar	ND	.0016	.004	.020
2.	Nagali	.006	.001	ND	.030
3.	Mehalka	.004	.006	.012	.020
4.	Kheri	.042	.009	.081	.189
5.	Mohammadpur	.098	.005	.942	.320
6.	Daurala	.34	.018	.342	4.72
7.	Iklauta	.12	.004	.148	.520
8.	Dhanju	.034	.001	.418	1.18
9.	Ajohata	.190	.031	.078	.165
10.	Dedwa	.283	.145	1.86	5.25
11	Jalalpur	.205	.03	1.54	.510
12.	Jalalpur	.185	.03	1.32	1.21
13.	Ulday Pur	.132	.02	1.43	1.81
14.	Ulday Pur	.131	.02	1.40	1.24
15.	Mathana	.110	.05	.560	1.45
16.	Khanauda	.146	.04	.022	.180
17.	Chalera	.018	.02	.118	.110
18.	Aurangabad	.099	.03	.198	1.65
19.	Aurangabad	.078	.009	.178	.150
20.	Senni	.148	.001	.017	1.82
21.	Rali	.100	.03	.042	.620
22.	GeshuPur	.420	.04	.134	3.52
23.	Kajipur	.220	.01	.121	1.25
24.	Gokul Pur	.142	.03	1.18	1.38
25.	Jai Bhim Nagar	.810	.04	7.18	2.62
26.	Jai Bhim Nagar	.721	.02	4.18	1.85
27.	Alipur	.102	.008	.089	.550
28.	Kohal	.100	.05	3.20	1.60
29.	Ataria	ND	.01	.280	1.62
30.	Itola	.056	.03	1.12	.940
31.	Ajrana	.048	.01	.450	.120
32.	Kudha	.094	.001	.480	10.28

Table 2. Heavy metal contents (average) in fertilizers commonly used in the study area. (Pathak *et al.*, 2002)

Fertilizer	Heavy metal (mg kg^{-} fertilizer)					
	Cu	Zn	Mn	Mo	Cd	Pb
Single super phosphate	26	115	150	3.3	187	609
Diammonium Phosphate	-	-	-	109	188	-
Muriate of potash	3	3	8	0.2	14	88
Ca-ammonium nitrate	0.2	6	11	-	6	200
Urea	0.4	0.5	0.5	0.2	1	4
Ammonium Sulphate	0.5	0.5	70	0.1	-	-
Triple super Phosphate	7	75	200	0.1	-	-
Ammonium Phosphate	3	80	160	2	-	-
Complex fertilizer	22	276	-	-	6	128
Rock Phosphate	100	200	0.5	-	-	-

RESULT AND DISCUSSION

Manganese occurs mainly in the form of maganese and mangniferrous oxide in ground water. It is obvious from the data (table 1) that the manganese was available in maximum samples and ranged varied from 0.006 to 0.81 mg/l. The manganese concentration was within the permissible limit in all the samples, in the study area. The maximum allowable concentration and the permissible concentration of manganese in drinking water are 0.5 and 1.0 mg/l, respectively, according to WHO, (1984) and ISI, (1983). Manganese is one of the most abundant metals in the earth's crust and usually occurs with iron. Edmunds and Smedley (2000). Edmunds (2000) suggested that manganese could be released by incongruent reactions from silicate or oxide minerals and emerged as potential residence-time indicators. Besides, some elements as redox-sensitive and local chemical conditions could affect their availability and mobility in groundwater. (Zachara *et al.,* 1995, Kedziorek *et al.,* 1998 and Davis *et al.,* 2000).

Copper was available in each sample from villages which varied from 0.001 to 0.14 mg/l. The concentration of copper was within permissible limit. The permissible limit of copper is (2mg/l) as per recommendation of WHO, (2004) and (2011). High concentration of copper in some samples in the study area might be due to interior copper plumbing. (USPEA, 1991). Copper is essential micronutrient, but its high concentration causes physiological effect in human being. Water containing 3mg/l was associated with gastrointestinal disturbance in adults (Pizarro et al 1999). Excess copper in human body is toxic and causes hypertension and produces pathological changes in brain tissues. However, excessive ingestion of copper is responsible for specific diseases of bone (Krishnamurthy and Pushpa, 1995)

Zinc concentration in ground water varied from 0.004 to 7.18 mg/l in the study area. The maximum allowable concentration and the permissible concentration of zinc in drinking water are 10 and 5.0 mg/l respectively, according to ISI (1981). The concentration of zinc is within the permissible limit in all samples. However, according to the guideline of EPA, approximately 59% water samples showed higher concentration than permissible limit. The permissible limit of zinc in drinking water is 1.5 mg/l according EPA guideline. Zinc occurs as a natural mineral in most of the drinking water. It is an essentially dietary nutrients and beneficial human health (Vallce, 1957). However, excess zinc concentration creates aesthetic effect (metallic taste in the water). In this study area, intensive agricultural activities high uses of fertilizers and micronutrients may be a major source of high concentration of zinc in groundwater. Similar results were reported by Rajmohan and Elango (2005) in groundwater of South India. In some samples, high concentration of zinc may be due to leaching of zinc from piping and fitting (Nriagu, C. 1980).

Zinc is nutritionally essential elements which is necessary for growth and is involved in several physiological functions. Nevertheless, at higher concentration, zinc can be toxic to the organism. It is also plays an important role in protein synthesis. Zinc is a metal which shows fairly low concentration in surface water, which is due to its restricted mobility from the place of rock weathering or from the natural sources (BIS, 1998).

Iron contamination in drinking water in the study area ranged between 0.02 to 10.28 mg/l. The maximum allowable concentration of iron in drinking water is 1.5 mg/l according to WHO, (2004) and (2011). As per ISI, the permissible limit of iron in drinking water is 0.3 mg/l. In the study area, 18 samples out of 32 samples showed higher concentration according to WHO standard and 22 samples showed, the concentration of iron more than permissible limit (BIS, 1995). It seems to be of high content of iron in 56% of samples and water can be used other than drinking purpose. The high concentration of iron in some samples might be due to a lot of iron industries, effluent of paper mills, sugar mills dumped in kali east river without any treatment. Another reason for its contamination may be due to rainwater percolating through soil and rocks dissolves minerals containing iron and hold this in solution. These iron rich water recharge surface water and aquifers that inevitably serve as drinking water sources (Colter and Mahler 2006). The higher concentration of iron in hand pump and tube wells water might be due to soil origin and age old corroded iron pipes. (Borah *et al*. 2009).

During the course of investigation, there are number of major and minor industries which discharge their effluents in leaked open channels, sewerage line from city and villages also dump their solid and liquid wastes to the kali east river which pollute the river and water resources around the study area. In the study area the major ion concentration were higher than that of ground water observed in the rest of the region. This may be due to continuous withdrawal of groundwater for drinking through bore-wells which leads to river water intrusion in the ground water.

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EFFECT OF TREATMENT WITH LEAD SULPHATE ON SOIL MYCOBIOTA

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Abstract: Nineteen species of fungi were isolated from control soils and that treated with lead sulphate solutions (20ppm, 40ppm, 100ppm and 250ppm) for 90 days. Treatment with lead sulphate did not result in substantial decrease in the number of species isolated. Greater number of isolates was obtained from Pb-treated soils except in general. The species which could tolerate higher concentration of lead sulphate for 90 days included *Aspergillus flavus, Aspergillus ustus, Aspergillus niger* and *Trichoderma lignorum*. *Aspergillus fumigatus* and *Botryotrichum piluliferum* exhibited remarkable resistance to lead as these dominated the soil treated with lead sulphate solution for 90 days.

Keywords: Heavy metals, Lead pollution, Metal tolerant fungi, Soil microflora.

INTRODUCTION

The pollution by heavy metals is amongst the major environmental problems (Dushenkov *et al.,* 1995; Piccardo *et al.,* 2009; Nawachukwu *et al.,* 2010). Considerable amounts of heavy metals, including lead, are introduced into aquatic systems as a result of mining, smelting, printing, battery-manufacturing, electroplating, tanning etc. Beyond a certain limit, these metals exert adverse effects on the environment and ultimately accumulate in living tissues through the food chain with human at its top. Hence, these metals need to be removed before these enter the complex ecosystem. Physico-chemical treatment procedures developed to deal with much diluted metal-containing effluents (precipitation, flocculation, coagulation, ion-exchange etc.) are very expensive (Lacina, 2003). The ability of fungi to serve as biotraps for heavy metals and biosorb them has attracted the attention of a number of workers for purification of such effluents (Gadd and Griffiths, 1978; Hemapriya *et al.,* 2010; Can and Jialong, 2010). The purification of the metal-containing water using fungal biomass is not only cheaper but also offers the following advantages: (i) fast removal, (ii) production of small residual volume, (iii) easy installation of the process, (iv) recovery of metals from the treatment columns. Soil constitutes an excellent reservoir of immense variety of fungi. It would be, therefore, pertinent to explore the possibilities of getting suitable fungal strains from soil which are capable of removing metals from the wastewater effectively. Out of many fruitful approaches for obtaining metal-resistant microbes, one is to treat the soil with given pollutant and to isolate microbes which are able to survive it (Antonovics *et al.,* 1971). The present communication deals with the effect of lead sulphate treatment on soil mycobiota with the aim to obtain the soil fungal strains capable of surviving lead sulphate pollution with an ultimate aim to utilize these for mitigating lead pollution.

MATERIAL AND METHOD

Forty five plastic pots of 150 ml capacity were filled with 100 gm soil each. These were divided into five sets of nine pots each. Nine pots of set 1 were treated with 25 ml of distilled water at regular intervals of 7 days for a total period of 12 weeks. This set served as control. The nine pots of set 2, nine pots of set 3, nine pots of set 4 and nine pots of set 5 were treated similarly but with 20 ppm, 40 ppm, 100 ppm and 250 ppm solutions of lead (as lead sulphate) respectively (instead of distilled water). A separate sub-sample was analyzed for soil mycobiota using dilution plate method (Waksman, 1927).

After 30 days, soils from three pots of set 1 were mixed thoroughly but aseptically to obtain a composite sample. Similar composite samples were obtained for set 2, 3, 4 and 5. Each composite soil sample so obtained was analyzed for mycobiota using dilution plate method. 20 grams of soil from a given composite sample were transferred to 20 ml of sterilized distilled water and stirred for 30 minutes to wash fungal propagules from the material. 10 ml of this suspension were immediately transferred to a conical flask containing 90 ml of sterilized distilled water. This suspension (1 : 100 dilution) was used for preparation of further dilution of 1 : 1000. From the suspensions of each of the 1 : 100 and 1 : 1000 dilution, one ml aliquots were transferred to each of a set of 3 Petri dishes followed by the addition of 20 ml of cooled and sterilized Potato-Dextrose Agar Medium (Raper and Thom, 1949) with 30ppm Rose Bengal and 30 mg of Streptomycin. The Petri dishes containing the medium and the inocula were incubated at 25 ^{o}C for 6−8 days. The total numbers of colonies of individual fungal species growing in each Petri dish were recorded. The fungal strains obtained were identified using standard keys (Gilman, 1957; Subramanian, 1971; Nagmani *et al.,* 2006).

RESULT AND DISCUSSION

In all, 19 species of fungi were isolated in the present study. The data concerning the total isolates (TI) and

percentage isolates (PI) of fungi obtained at the beginning of the experiment as also after 30 days, 60 days and 90 days for control as well as various treatments are presented in the tables 1 to 3. Out of the nineteen species, only one belongs to Zygomycota. The remaining eighteen species were anamorphic fungi (Deuteromycota). The dominance of Deuteromycota observed in the present study is in full agreement with the earlier reports (Hudson, 1968; Dickinson and Pugh, 1974; Hayes and Lim, 1979; Charaya, 2006; Tiwari and Charaya, 2006; Sen and Charaya, 2010). Only one member belonging to the order Mucorales was isolated in the present study, thus, supporting the findings of Galloway (1935), Singh and Charaya (1975), Dube *et al*. (1980), Singh (2004) and Charaya (2006) which indicated that there is paucity of mucorales in the tropical regions of India. Among the Deuteromycota, the Hyphomycetes constituted the major component. Nine species belonging to Moniliaceae and seven species belonging to the Dematiaceae were obtained. Among the Moniliaceae, six species belong to the genus *Aspergillus* while only two belong to genus *Penicillium*. It is widely believed that Aspergilli are more common in the warmer regions of the world while the Penicillia are more abundant in the colder regions. The results of the present study as also those carried out by a number of workers (Waksman, 1917; Jensen, 1931; Singh and Charaya, 1975; Charaya, 2006; Sen *et al.*, 2009) support the aforementioned generalization.

Out of initial isolates, eight fungal species i.e. *Alternaria alternata, A. citri, Aspergillus luchuensis, Cladosporium herbarum, Curvularia lunata, Fusarium* sp., *Helminthosporium nodulosum*

Table 1. Mycobiota isolated from control soil as well as that amended with lead sulphate (20ppm, 40ppm, 100ppm and 250ppm) after 30 days of treatment

Fungal Species	**Initial**		**Control**		**Treatment**							
					20 ppm		**40 ppm**		**100 ppm**		**250 ppm**	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Alternaria alternata	4	2.38	-	-	-	-	-	-	-	-	-	-
Alternaria citri	2	1.19	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	22	13.1	4	2.58	1	0.52	1	1.07	5	8.06	5	4.23
Aspergillus fumigatus	53	31.5	29	18.70	16 6	87.83	67	72.0 4	26	41.93	87	73.72
Aspergillus luchuensis	8	4.76	-	-	-	-	-	-	-	-	-	-
Aspergillus niger	18	10.71	20	12.90	7	3.70	10	10.7 5	7	11.29	14	11.86
Aspergillus terreus	-	-	3	1.93	2	1.05	2	2.15	-	-	1	0.84
Aspergillus ustus	13	7.73	10	6.45	-	-	-	-		8.06	1	0.84
Botryotrichum atrogriseum	-	-	-	-	2	1.05	1	1.07	1	1.61	-	-
Botryotrichum piluliferum	19	11.30	85	54.83	9	4.76	11	11.8 2	16	25.80	9	7.62
Cladosporium herbarum	3	1.78	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	1	0.59	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	3	1.78	-	-	-	-	-	-	-	-	-	-
Helminthosporium nodulosum	1	0.59	-	-	-	-	-	-	-	-	-	-
Penicillium implicatum	7	4.2	-	-	-	-	-	-	-	-	-	-
Penicillium sp.	-	-	2	1.29	-	-	-	-	-	-	-	-
Rhizopus sp.	6	3.57	-	-	1	0.52	-	-	-	-	-	-
Trichoderma lignorum	-	-	1	0.64	1	0.52	1	1.07	1	1.61	-	-

White sterile mycelia	8	4.76	1	0.64	-	-	-	-	1	1.61	1	0.84
No. of Species	15	-	9	-	8	-	7	-	8	-	7	-
Total Isolates	168	-	155	-	189	-	93	-	62	-	118	-

TI= Total isolates; PI= Percentage of isolates

and *Penicillium implicatum* could be isolated from the initial sample (collected from the field itself) only, and were not isolated subsequently even from the control samples (placed in pots). Obviously, the soil might have got substantially disturbed during transfer from field to pots–causing alterations in resource availability and system structure (Pickett and White, 1985); leading, in turn, to a decrease in species richness and system heterogeneity (Zak, 1992). Again, out of these eight species, six species *viz. A. alternata*, *A. citri*, *C. herbarum*, *C. lunata*, *Fusarium* sp. and *Helminthosporium nodulosum* were meagerly represented. It is quite possible that these six species might have been allochthonous to soil collected from the field and disappeared rapidly (Atlas and Bartha, 1998). The disturbance in soil seems to have an adverse effect on the populations of all other fungal species also with the exception of *Botryotrichum piluliferum* whose population in control samples exhibited steep increase over initial sample. *Penicillum* sp. and *Trichoderma lignorum* were the two species which were not isolated from the initial sample but were obtained from subsequent samples.

The treatment of soil with lead sulphate did not appear to have appreciable influence on the number of species isolated. The number of species isolated from soils treated with different concentrations of lead sulphate were either equal to those isolated from control soil or only one/two species lesser than that. As far as the numbers of isolates are concerned, more isolates were obtained from the soils treated with lead sulphate as compared to those obtained from the control samples.

Table 2. Mycobiota isolated from control soil as well as that amended with lead sulphate (20ppm, 40ppm, 100ppm and 250ppm) after 60 days of treatment.

Fungal Species	**Initial**		**Control**		**Treatment**							
					20 ppm		**40 ppm**		**100 ppm**		**250 ppm**	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Alternaria alternata	4	2.38	-	-	-	-	-	-	-	-	-	-
Alternaria citri	2	1.19	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	22	13.1	8	5.40	7	2.75	3	1.22	49	21.68	8	4.90
Aspergillus fumigatus	53	31.5	39	26.35	157	61.81	48	19.59	89	39.38	82	50.30
Aspergillus luchuensis	8	4.76	-	-	-	-	-	-	-	-	-	-
Aspergillus niger	18	10.71	25	16.89	12	4.72	12	4.89	21	9.29	17	10.42
Aspergillus terreus	-	-	-	-	1	0.39	-	-	-	-	-	-
Aspergillus ustus	13	7.73	-	-	-	-	8	3.26	2	0.88	1	0.61
Botryotrichum atrogriseum	-	-	-	-	-	-	-	-	-	-	-	-
Botryotrichum piluliferum	19	11.30	70	47.29	68	26.77	170	69.38	53	23.45	52	31.90
Cladosporium herbarum	3	1.78	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	1	0.59	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	3	1.78	-	-	-	-	-	-	-	-	-	-
Helminthosporium nodulosum	1	0.59	-	-	-	-	-	-	-	-	-	-
Penicillium implicatum	7	4.2	-	-	-	-	-	-	-	-	-	-
Penicillium sp.	-	-	2	1.35	1	0.39	-	-	3	1.32	2	
Rhizopus sp.	6	3.57	-	-	-	-	-	-	-	-	-	-
Trichoderma lignorum	-	-	2	1.35	-	-	-	-	-	-	-	-

White sterile mycelia	8	4.76	2	1.35	8	3.14	4	1.63	9	3.98	3	1.84
No. of Species	15	-	7	-	7	-	6	-	7	-	6	-
Total Isolates	168	-	148	-	254	-	245	-	226	-	163	-

Konopka *et al.* (1999) reported that the populations of culturable microbes were lower in lead contaminated soils. On the contrary, McGrath (2001) suggested that lead is insoluble in soils and, therefore, it is unlikely to be bioavailable; consequently it does not have any adverse effect on soil microbiota. Sen *et al.* (2009) observed that the addition of lead as lead nitrate to the culture medium upto 200 ppm concentrations did not have much effect on the number of species isolated from the soil, though at 400 ppm lead concentrations, the number of species as well fungal population were markedly reduced. On the other hand, Tiwari (2010) used 200 ppm and 500 ppm solutions of lead as lead nitrate and recorded substantial reduction in the total number of fungal isolates as well as mycodiversity in lead nitrate-treated soils as compared to the control soils. In the present study, 20 ppm, 40 ppm, 100 ppm and 250 ppm solutions of lead sulphate were used. The results of the present study agree to a marked extent with that of Sen *et al.* (2009) in that she also failed to record any adverse effect on mycodiversity upto 200 ppm concentrations with lead nitrate. A number of reports (Hutchinson, 1973; Smith, 1977; Henriksson and DaSilva, 1978; Sen *et al.*, 2009) exist regarding the stimulatory effect of lead pollution on microorganisms and their activity. Sen *et al.* (2009) reported that 40 ppm concentration of lead resulted in noticeable stimulatory effect on the radial growth of *Aspergillus candidus*, *A. niger*, *Penicillum implicatum*, *Sepedonium chrysospermum* and *Trichoderma lignorum*. Kumar (2011) demonstrated that the *in vitro* growth of *Aspergillus fumigtus* and *A. niger* were stimulated by lead sulphate upto 800 ppm. Increase in the fungal population with low concentrations of lead sulphate after 30 days of treatments, in the present study, is an agreement with the observations of above cited workers to a considerable extent.

Table 3. Mycobiota isolated from control soil as well as that amended with lead sulphate (20ppm, 40ppm, 100ppm and 250ppm) after 90 days of treatment.

Fungal Species	**Initial**		**Control**		**Treatment**							
					20 ppm		**40 ppm**		**100 ppm**		**250 ppm**	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Alternaria alternata	4	2.38	-	-	-	-	-	-	-	-	-	-
Alternaria citri	2	1.19	-	-	-	-	-	-	-	-	-	-
Aspergillus flavus	22	13.1	11	8.66	16	8.98	1	0.40	59	22.77	7	4.29
Aspergillus fumigatus	53	31.5	37	29.13	59	33.14	56	22.95	104	40.15	53	32.51
Aspergillus luchuensis	8	4.76	-	-	-	-	-	-	-	-	-	-
Aspergillus niger	18	10.71	3	2.36	6	3.37	5	2.04	10	3.86	4	2.45
Aspergillus terreus	-	-	-	-	2	1.12	-	-	-	-	-	-
Aspergillus ustus	13	7.73	6	4.72	3	1.68	11	4.50		1.54	3	1.84
Botryotrichum atrogriseum	-	-	-	-	-	-	-	-	-	-	-	-
Botryotrichum piluliferum	19	11.30	65	51.18	87	48.87	161	65.98	71	27.41	79	48.46
Cladosporium herbarum	3	1.78	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	1	0.59	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	3	1.78	-	-	-	-	-	-	-	-	-	-
Helminthosporium nodulosum	1	0.59	-	-	-	-	-	-	-	-	-	-
Penicillium implicatum	7	4.2	-	-	-	-	-	-	-	-	-	-
Penicillium sp.	-	-	2	1.57	-	-	-	-	-	-	-	-
Rhizopus sp.	6	3.57	-	-	-	-	-	-	-	-	-	-
Trichoderma	-	-	3	2.36	-	-	-	-	-	-	1	0.61

lignorum												
White sterile mycelia	8	4.76	-	-	5	2.80	10	4.09	11	4.24	16	9.81
No. of Species	15	-	7	-	7	-	6	-	6	-	7	-
Total Isolates	168	-	127	-	178	-	244	-	259	-	163	-

Another interesting result obtained in the present study is that as the number of days increased (Tables 2 and 3), the fungal populations in the soils treated with higher concentrations of lead sulphate were more than that in control soils. Babich and Stotzky (1982) suggested that the levels of a pollutant which are lethal to a majority of microbes may only cause mutation in some and thereby increase the selection of the strains which can tolerate the higher concentrations of the pollutant. The subsequent survival and multiplication of these strains might have lead to an increase in the populations of such strains resulting in a total positive effect on the fungal population.

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STRUCTURE AND PHENOLOGY OF AN ALPINE MEADOW AS AFFECTED BY NOMADIC GRAZING

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Abstract: Data were collected for structure and phenology of alpine grassland at Rudranath in Uttarakhand, India. A large number of species of the area are dwarf cushion herbs and most are distributed in mid alpine tract. A total of 21 and 16 species were recorded at control (S1) and grazed (S2) plot respectively. At control or ungrazed site maximum density (211.0 pl/m^2) and basal cover (121.6 pl/m^2) was recorded for *Danthonia cachyymeriana* and for grazed plot maximum density (146.0 pl/m^2) and basal cover (170.9 pl/m^2) was for *Oxygraphis polypatela.* In most of the cases, the various species completes their life cycle within 4-5 months. Germination of various species starts during April-May with luxuriant vegetative growth. Majority of species bear flowers during July and August. Some species bear flowers during later part of June. Seed formation begins in later part of August and increase sharply up to September. Senescence at community level is gradual from September and increases quickly due to lower temperature. Thereafter different phenophases succeeded one after the other and completed their life history up to November.

Keywords: Phenophases, Phenology, Sprouting, Senescence, Vegetative

INTRODUCTION

The alpine zone occupy nearly 33% of total geographic area in the Himalaya, of which the vegetative and snow bound areas constitute about 25.88% and 7.22% respectively (Anonymous, 1989). Bounded by a distinct tree line towards lower elevation, that varies from 3300±200 m in the west to 3800±200 m in the east. The alpine vegetation comprises closely woven strands of scrubs, meadows, bogs and fell fields paved with mosses and lichen. Numerous environmental factors govern the structure and function of these communities (Puri et al., 1989).

The landscape lying between the tree line and glaciers is known as high altitude meadows (Bughyals); represent the alpine zone of great Himalaya where vegetation consists of short stemmed perennial herbaceous plants, semi–prostrate shrubs, ferns, lichens and mosses (Ram and Singh, 1994). The total geographic area of this zone in Central Himalaya is 13528 sq.km., out of which only 1840 sq.km. is covered by alpine vegetation and rest of the area is characterized by permanent snow cover (Tewari *et al.,* 1985). These meadows have been subjected to migratory nomadic grazing during snow free period (May to October). Vegetation in the alpine zone exhibits a characteristic adaptation to the environment. They possess an early growth initiation with a short vegetative span ranging from several days to a few months. The community as a whole usually exhibits seasonal fluctuations, and its structure and composition are strongly influenced by the extent to which periodic phenomena in the individuals are adjusted to each other. Therefore, in pursuance of structural and functional attributes of ground vegetation in alpine meadows, knowledge of different phenophases for individual species is imperative to understand the complexity of the system. In addition, the life forms of species represent the adjustment of perennating organs and plant life history to environmental conditions, an important characteristic in describing vegetation that offers a preliminary picture of the ecological character of the vegetation (Kershaw 1973). A great deal of work has been done on the alpine communities of Garhwal (Semwal *et al*., 1981; Sundriyal et al 1987; Ram and Arya 1991; Nautiyal *et al*., 1997). But there is little information on the phenology and growth form distribution of alpine vegetation at the community level. The present article examines the structure and phenological response of different species of flowering plants in a variety of microhabitats in Rudranath in relation to growth cycle, growth initiation period, timing of flowering and fruiting, and life form distribution.

Study area

The present study was carried out in 2005 at Rudranath in Chamoli District of Uttarakhand of India. The area lies in between 30^0 05' North latitude and 79^0 07' East longitudes, in the North of Uttarakhand at an elevation range of 3300 to 4500 m above msl. The growing season of this zone starts from mid April and ends in mid November (About 200 days). The area remains snow covered in rest of the period. The average temperature does not exceed 20^0 c in June and it recedes to 1.5^0 c in first week of November. Maximum precipitation was recorded in August (450 mm) with a total of 2100 mm rainfall during growing season. Geologically, the area is a complex system of Tertiary Mountain which is nearly parallel and well kwon for having beautiful

landscape. The palaeozoic age rocks are crystalline and metamorphic in nature with sedimentary deposits. The soil of the study area was blackish brown in colour and the soil moisture remains high (over 40%) throughout the growing season. All the soils were slightly basic (ph 7.52), with a carbon percentage of 0.342%. The percentage of nitrogen, phosphorus and potash was found fairly high due to accumulation and slow decomposition of organic matter.

METHODOLOGY

The field study was conducted at Rudranath alpine meadow at an elevation of 3500 m msl during 2009. One plot protected from any type of grazing and treated as control (S1). The adjacent area remained open for all type of grazing and treated as grazed plot (S2). The area was surveyed for several times during the study for extensive plant collection. Collected species were assigned to various life from classes according to the system proposed by Raunkiaer (1934). The phenological data were recorded during each month from 15th April to 15th November, 2009. Five phenophases, *viz*. germination of seeds and sprouting of underground parts (seedlings of 0.2 cm in height in monocots and up to first leaf stage in dicots), vegetative phase, flowering phase, fruiting phase and senescence phase were noted for all collected species.

Community structure of both the sites was determined by laying 50 quadrat of 25 x 25 cm size randomly in both the study sites, covering all the possible slopes and directions. The size of the quadrat was determined by the species area curve method following Misra, 1968. The phytosociological data were quantitatively analyzed for density, abundance and basal cover of each species following the method given by Curtis and McIntosh (1950).

RESULT

A Perusal of results of presence and absence of different species reveals that a total of 21 and 16 species were present at control and grazed plot respectively (Table 1). Asteraceae was the leading family in the area (23 %) followed by Poaceae and Rosaceae (13%) each. Life forms analysis of different species shows that therophyte, chaemophyte and hemi-cryptophytes were 26.08% and geophytes were 21.7% while phanerophytes were found absent in alpine meadows (Table 1).

The results of phytosociological analysis are presented in Table 2. Maximum density was noted for *Denthonia cachymeriana* (211.0 pl/m^2) followed by *Trachydium roylei* (155.7 pl/m^2) at control plot. *Oxygraphis polypetala* represent maximum density at grazed plot (146.00 pl/m^2). However, the minimum density at control plot was found for *Jurinea macrocephala* (0.3 pl/m^2) and *Bupleurum lanceolatus* (1.2 pl/m^2) respectively. Analysis of total basal cover indicates that maximum basal area was covered by *Danthonia cachymeriana* (121.6 cm^2/m^2) at control plot and by *Oxygraphis polypetala* (170.9 cm^2/m^2) respectively. Analysis of A/F ratio indicates regular and random distribution of species in both the plots.

Phenological observations indicate that germination of seed and sprouting of rootstocks started with the melting of snow and rising of temperature during April and May. The growth initiation peaked in June when the day temperature in the open averaged 16^{0}c. Majority of species bear flowers during July and August. Some species bear flowers during later part of June. Seed formation begins in later part of August and increase sharply up to September. Senescence at community level is gradual from September and increases quickly due to lower temperature. Thus in the high altitude meadows, the peak of the various phenophases succeeded one after the other but they occurred in very short period of 4 - 5 months.

Germination of maximum species was recorded in April (47.8%). Maximum vegetative phase of different species was during June (78.26%). Flowering (65.21%) and fruiting (43.47%) was represented by maximum number of species in August and September respectively. Senescence followed the fruiting season and attain by maximum species during November (65.21%). Yellow coloured flowers were found in maximum no. of species (26.08%) and then the white flowers (21.73%).

DISCUSSION

Due to its geographical location between Himalayas and Zanskar range, Rudranath shows high landscape diversity and resultant plant species diversities. In Rudranath, the highest number of plant species represents Asteraceae followed by Roasaceae and Poaceae. In a comparative study between flora of valley of flowers and flora of district Chamoli, Naithani (1984) reveals that Asteraceae possesses first place in both the flora. Kala *et al.* (1998) also found the similar results in Nanda Devi Park flora.

The biological spectrum of the present study site and other biological spectrum of India have been compared with Raunkiaer's normal spectrum. Highest number of the therophytic and hemicryptophytic flora indicates shorter life cycle and mode of perennation due to peculiar environmental conditions at alpine zone (Rikhari and Negi, 1994) and higher influence of human and cattle interaction (Barucha and Dave, 1944). (Cain, 1950) was of the view that a high percentage of therophytes in grasslands was due to overgrazing.

Topography, climate, edaphic factors and aspects influence the growth and establishment of high altitude flowering plants. These species exhibit a

strong seasonality and temporal separation from each other (Kala *et al.,* 1995). The total vegetation density, as well as density of individual species together with the basal cover, fluctuated throughout the study period in response to environmental variation and grazing pressure. The pattern of change in density differs from species to species and shows variable response to growing conditions. Identical results have been observed by Singh and Yadava (1974) and Kala *et al.* (1998). Lower density of some plant species in the grazed plot attribute to their lower resistance towards grazing. High density of *Oxygraphis polypetala* at grazed plot shows that they thrive better in grazing conditions. Change in density due to grazing was also reported by Singh *et al.* (1980).

The relationship between frequency and abundance (Whitford, 1949) revealed that majority of species were randomly and regularly distributed on both the sites. Climatic transition and site conditions affected the distribution of species. According to Odum (1971) random distribution is found only in uniform environment and regular distribution occurs where competition between several individuals exists. The present community also showed contagious distribution which may be due to extensive grazing.

Phenology embraces all studies of relationship between climatic factors and periodic phenomenon of organisms. Seasonal progression in life cycle of different species and periodicity of the various stages varies considerably. This can be correlated with species and environmental reactions after the snow melting, spring air, soil temperature etc. (Sundriyal *et al.,* 1987). Germination, sprouting, vegetative growth, flowering, seed maturation and dormancy induction appear to be directly regulated by absolute or relatively soil temperature. Shading and nutrient availability may be other important factors controlling the germination and sprouting of buds.

In alpine meadows of the Himalayan region, some species grow early while other grows later in the growing season. This is attributed to differences in capacity to absorb water at low temperature and may perhaps be correlated to higher level of soluble carbohydrate (Mooney and Billings, 1961). Flowering of species also varies due to photoperiodic response at high elevation. Temperature is the most important factor for controlling the plant activities (Holway and Ward, 1965). Production of high carbohydrates in some instances also results in development and production of new buds in late season. The late flowers may have resulted from the bolting of floral axis that normally would have been carried in to the next growing season in the perennating organs (Holway and Ward, 1965).

Flowering was followed by fruit development, which is species dependent in most cases. Fruits developed in 3-5 weeks after flowering. Most of the species were found turning dry at the end of October as the result of onset of severe climatic conditions, continuous frost, and sharp decline in temperature and occasional snowfall that prevent further plant growth (Sorenson, 1941).

Active plant growth started with the rising temperature and snow melting (April-May). Vigorous growth of tillers from perennial belowground plant parts initiated which followed high growth rate.

Table 1. Presence (+), Absence (-) and Life Forms of Different Species at Site 1 and 2

S. No.	Name of Species	S1	S2	Life Forms
1	*Agrostis stolonifera* Linn	+	-	G
2	*Anaphalis royleana* D.C.	+	-	Th
3	*Bupleurum lanceolatum* Wall ex D.C.	-	+	Th
4	*Campanula argerotricha* Wall ex D.C.	+	+	Ch
5	*Danthonia cachemyriana* Jaub and Spach.	+	+	G
6	*Dorydalis cashimeriana* Royle	+	-	G
7	*Epilobium royleanum* Haussk.	+	-	G
8	*Geranium wallichianum* D.Don ex Sw	+	+	Ch
9	*Geum elatum* (Royle) Hk.F	+	+	Hcr
10	*Impatience roylei* Walp. Rep.	+	+	Th
11	*Jurinea macrocephala* (Decne) C.B. Clarke	+	+	Ch
12	*Juncus trifidus* L.	+	+	Hcr
13	*Kobressia nitens* C.B. Clarke	+	-	G
14	*Oxygraphis polypetala* H.F and T.Fl.	-	+	Th
15	*Parnessia nubicola* Wall ex Royle	+	-	Ch
16	*Polygonum macrocephyllum* D.Don	+	+	Ch
17	*Polygonum vaccinifolium* Wall ex Meissn	+	+	Ch
18	*Potentiella astrsanguinea* Lodd.	+	+	Hcr
19	*Saxifraga diversifloia* Wall ex Dc.	+	+	Th
20	*Sibbaldia perviflora* Willd.	+	-	Hcr

21	*Sausurea taraxacifolium* Wall ex D.C.	+	+	Hcr
22	*Tenacetum longifolium* Wall ex D.C.	+	+	Hcr
23	*Trachydium roylei* (Edgew.) Clarke	+	+	Th

Fig. 1 Phenological pattern of different species found in the study area during the study year

Name of Species	Apr	May	June	July	Aug	Sep	Oct	Nov
Agrostis stolonifera Linn								
Anaphalis royleana D.C.								
Bupleurum lanceolatum Wall ex D.C.								
Campanula argerotricha Wall ex D.C.								
Danthonia cachemyriana Jaub and Spach.								
Dorydalis cashimeriana Royle								
Epilobium royleanum Haussk.								
Geranium wallichianum D.Don ex Sw								
Geum elatum (Royle) Hk.F								
Impatience roylei Walp. Rep.								
Jurinea macrocephala (Decne) C.B. Clarke								
Juncus trifidus L.								
Kobressia nitens C.B. Clarke								
Oxygraphis polypetala H.F and T.Fl.								
Parnessia nubicola Wall ex Royle								
Polygonum macrocephyllum D.Don								
Polygonum vaccinifolium Wall ex Meissn								
Potentiella astrsanguinea Lodd.								
Saxifraga diversifloia Wall ex Dc.								
Sibbaldia perviflora Willd.								
Sausurea taraxacifolium Wall ex D.C.								
Tenacetum longifolium Wall ex D.C.								
Trachydium roylei (Edgew.) Clarke								

Table 2. Density, A/F and Total Basal Area of Different Plant Species at Site 1 and 2

S.No.	Name of Species	Site 1			Site2		
		D	A/F	TBA	D	A/F	TBA
1	*Agrostis stolonifera*	16.00	0.29	3.01	-	-	-
2	*Anaphalis royleana.*	12.90	0.21	0.07	-	-	-
3	*Bupleurum lanceolatum*	-	-	-	1.20	0.29	0.42
4	*Campanula argerotricha*	4.20	0.13	4.00	1.60	0.09	4.38
5	*Danthonia cachemyriana*	211.00	0.36	121.60	129.00	0.34	74.30
6	*Dorydalis cashimeriana*	9.20	0.05	1.52	-	-	-
7	*Epilobium royleanum*	4.70	0.11	4.47	-	-	-
8	*Geranium wallichianum*	4.80	0.21	2.96	3.60	0.19	2.22
9	*Geum elatum (Royle)*	6.50	0.07	2.31	2.20	0.03	0.78
10	*Impatience roylei*	4.20	0.09	1.49	1.80	0.06	0.64

11	*Jurinea macrocephala*	0.30	0.31	0.09	2.80	0.27	0.90
12	*Juncus elegans*	7.70	0.22	0.68	3.70	0.29	0.63
13	*Kobressia nitens*	6.80	0.19	3.38	-	-	-
14	*Oxygraphis polypetala*	-	-	-	146.00	0.17	170.9
15	*Parnessia nubicola*	9.30	0.14	2.46	-	-	-
16	*Polygonum macrocephyllum*	14.30	0.19	6.05	7.30	0.22	3.09
17	*Polygonum vaccinifolium*	16.20	0.01	3.85	4.90	0.03	1.16
18	*Potentiella astrsanguinea*	9.20	0.12	2.98	1.80	0.19	2.33
19	*Saxifraga diversifloia*	14.60	0.01	3.87	7.20	0.11	0.47
20	*Sibbaldia perviflora*	12.20	0.19	5.16	-	-	-
21	*Sausurea taraxacifolium*	13.30	0.12	3.16	8.20	0.12	3.16
22	*Tenacetum longifolium*	32.00	0.11	30.49	7.60	0.04	7.24
23	*Trachydium roylei*	155.70	0.19	19.30	119.00	0.19	19.30

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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF BARK OF *LITCHI CHINENSIS* AGAINST *ESCHERICHIA COLI*, A UTI CAUSING ORGANISM

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Abstract: Main focus of present study was to screen the UTI patients, classification of patient on the basis of sex, age and antimicrobial activity of different ethanol, aqueous extracts of bark of *Litchi chinensis* L. against *Escherichia coli*. Agar well diffusion method was used to evaluate antibacterial activity against *E. coli*. Result suggested that Ethanol extract of *Litchi chinensis* showes more antibacterial activity as compared to aqueous extract, and norfloxacin against *E. coli*. On the basis of microbial count in urine sample, 30 out of 97 suspects were UTI positive. 70% females were UTI positive. Most infections were seen in age group of 16-30yr in both male (13.3%) as well as female (30%). Ethanol extract (30mg/ml) showed 31.86% more inhibition zone as compared to norfloxacin (30mg/ml). Aqueous extract (30mg/ml) also showed 23.56% more inhibition zone as compared to norfloxacin (30mg/ml)

Keywords: *Litchi chinensis*, Antibacterial, *E. coli*, UTI

INTRODUCTION

India enjoys the privilege of having time tested traditional system of medicines based on the natural products. Plants are being used as medicines by mankind since the ancient times and they are being taken as a good source of drugs (Deshwal and Siddiqui, 2011a). The word Ayurveda is derived from *Ayus* (r) meaning life, and *Veda*, meaning knowledge, thus Ayurveda literally means science of life and it is the ancient Indian system of healthcare and longevity (Sukh dev, 2006). Development of antibiotic resistant strains has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents (Singh *et al.,* 2010). Urinary tract infections (UTIs) are serious problems (Jacobsen *et al.,* 2008). Uropathogenic *Escherichia coli* (UPEC) is the most common etiologic agent, responsible for 80 to 85% of community- acquired UTIs (Ronald *et al.,* 2001).

The lychee (Litchi chinensis), a member of the Sapindaceae family, has its origin in China and is now widely spread in the tropical and subtropical regions of the world (Menzel, 1983). The lychee is one of the best fruit trees growing in the subtropics. Bhoopat *et al.* (2011) explained the antioxidant properties of the Gimjeng and Chakapat lychees as evidenced by the vitamin C and phenolic compounds, anti-lipid peroxidation and anti-apoptosis could explain the hepatoprotective effects in CCl(4)-induced hepatotoxicity. Besra *et al*. (1996) conducted pharmacological studies on *Litchi chinensis* and observed that extract was found to possess antiinflammatory, analgesic and antipyretic activity. Deshwal and Vig (2011) reported that Aqueous, ethanol and chloroform extracts of *Tribulus terrestris* seeds showed more inhibition zone as compared to norfloxacin. So, aim of present study was to screen the UTI patients, classification of patient on the basis of sex and age and antimicrobial activity of different ethanol, aqueous extract of bark of *Litchi chinensis* L. against *Escherichia coli*.

MATERIAL AND METHOD

Isolation of microorganism: Urine sample was collected in a sterilized container from a patient suffering from urinary tract infection. The mid-stream urine was collected after carefully cleaning the genitalia and mid-stream urine was collected because the first portion of urine may contain most of contaminants. Pathogens were isolated and counted by standard plate method and MacConkey agar without crystal violet and Blood agar medium was used for isolation of various pathogens. The plates were incubated at 37^{o}C for 24-48h.

Characterization of pathogens: Thirty suspects out of 97 samples were UTI positive and were analyzed for present study. Pathogens were characterized according to Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Preparation and selection of different extracts: Two extracts such as aqueous extract, ethanol extract were selected for present study:

(a) Preparation of Aqueous extracts: 100g dried finely powdered bark of plant were infused in distilled water until completely exhausted. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated and dried using rotary evaporator at 60°C. The final dried samples were stored at low temperature.

(b) Preparation of Ethanol extracts: Dried bark of plant was grounded and extracted in a percolator with 95% ethanol. About 10ml of ethanol per gram of sample was used. The ethanol extract was dried

under reduced pressure at 40°C. The dried extract was stored in sterile bottles for further use.

Sterilization and preparation of different concentration of extract: The dried extracts were exposed to ultra violet light (UV rays) for 24h to sterilize (Ekwenye and Elegalam, 2005). Liquid extracts were sterilized using a membrane filter (0.2µm sterile filter). Dry powder extracts were initially dissolved in 1ml of dimethyl sulfoxide (DMSO). Different dilutions of extract were prepared. Norfloxacin antibiotic worked as control drug.

Antibacterial activity of plant extract: Antibacterial activity was performed according to Deshwal and Vig (2011a). The microorganism was activated by inoculating a loopful of the strain in nutrient broth (30ml) and incubated on a rotary shaker. Then 0.2ml of inoculum (inoculum size was 10^8 cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the Petri plate. For agar well diffusion method, a well was made in the seeded plates with the help of a sterilized cup-borer. 20µl test compound was introduced into the well and the plates were incubated at 37ºC for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition.

RESULT AND DISCUSSION

On the basis of microbial count in urine sample, 30 out of 97 suspects were UTI positive. Isolated strains were characterized on the basis of Biochemical test and founded that strains were of *Escherichia coli*. Similar observation has been mentioned in Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

UTI patients were divided into age and sex. Four group of age were formed and these were 1-15yr, 16-30yr, 31-45yr and grater then 45. In all case, 70% females were UTI positive. Most infection was seen in 16-30yr in both male (13.3%) as well as female (30%) (Table-1). Our results showed that isolated strains were *E. coli* and infection was more common in female as compared to male. It could be possible because in females, the urethra is short and the area around the urethral opening is densely colonized with potential pathogens that make the females susceptible to UTI. Other scientists also observed the same results (Williams *et al.,* 2006; Hansson and Jodal, 2004).

Further, present study showed that bark of *Litchi chinensis* showed antibacterial activity. Antibacterial activity of water extract of *Litchi chinensis* was tested against *E. coli* culture at different concentration. The various concentrations of water extract of *Litchi chinensis* that were used are 15mg/ml, 20mg/ml, 25mg/ml and 30mg/ml. Ethanol extract showed more inhibition zone as compared to aqueous extract and norfloxacin antibiotics. Ethanol extract (30mg/ml) showed 31.86% more inhibition zone as compared to norfloxacin (30mg/ml). Aqueous extract (30mg/ml) also showed 23.56% more inhibition zone as compared to norfloxacin (30mg/ml) (Table 2). Similarly**,** Ekwenye and Elegalam (2005) reported that ethanolic extract of *ginger* (*Zingiber Officinale*) inhibited growth of *E. coli*. Further, Parekh *et al*. (2005) reported that methanolic extract of medicinal plant extract was effective against 5 medically important bacterial strains. Many researchers observed that extract of medicinal plants inhibited the growth of human pathogenic bacteria (Indul *et al*., 2006; Nair and Chanda, 2007; Al-Bayati and Al-Mola, 2008; Elekwa *et al.*, 2009; Deshwal and Vig, 2011a). Getachew *et al.* (2011) observed antibacterial activity of volatile fractions from *Artemesia abyssinthium*, *Croton macrostachyus*, *Echinops kebericho* and *Satureja punctata* tested against selected Gram-positive and Gram-negative bacterial strains. Recently, Gillitzer *et al*. (2012) reported the antimicrobial activity of *Betula papyrifera* and *Rhus typhina* against various pathogens.

All above facts and figures suggested that water and ethanol extract of *Litchi chinensis* inhibited the growth of *E. coli*. Further, other researches suggested that medicinal plants are good substitute of chemical drugs.

Table 1. Distribution of patients according to age and sex

S.No.	Age (years)	Sex	
		Male	**Female**
1	1-15	1	4
2	16-30	4	9
3	31-45	3	6
4	>45	1	2
Total		**9**	**21**

Table 2. *In vitro* antibacterial activity of water, ethanol extract of *Litchi chinensis* on the growth of *E. coli*.

S.No.	Concentration	Inhibition zone*		
		Litchi chinensis		Norfloxacin
		water extract	ethanol extract	

1	15 mg/ml	16.1 ±0.8mm	17.5±0.5mm	15.2±0.4mm
2	20 mg/ml	19.3±0.4 mm	20.8±0.6mm	16.5±0.8mm
3	25 mg/ml	22.5±0.5mm	23.6±0.5mm	18.3±0.5mm
4	30 mg/ml	25.2±0.4mm	26.9±0.5mm	20.4±0.4mm

*Values are mean of 3 replicate ± SD

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EFFICIENCY OF UNTREATED AND TREATED DAIRY EFFLUENT ON PHYSICO-CHEMICAL PROPERTIES OF THE SOIL

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Abstract : Samples of untreated and treated dairy effluent were collected from Parag milk plant, Meerut. Three concentrations (25, 50 and 100%) were used in this study. Tap water served as control. It was observed that soil pH decreased non-significantly in all the treatments with effluent application as compared to control. However, Nitrogen, Phosphorus and Potassium content of the soil increased significantly. Thus soil fertility improved in Integrated Nutrient Management System (I.N.M.S.) and agro-ecosystem.

Keywords : *Brassica juncea*, Meerut, Nitrogen, Phosphorus, Potassium

INTRODUCTION

Rapid industrialization has left with us polluted air, green house gases, choked rivers, contaminated soil, depleted ground water, ozone layer, global warming, El-Nino and La-Nino effect, endangered wild life and exhausted natural resources. Industries generate millions liters of waste water or effluent which are hazards to environment and deteriorate the quality of surface and ground water too (Ali khan and Dhaka, 1989, Ali Khan, 1990) and had adverse effect on alpha (α), beta (β), gamma (γ), biodiversity and biogeographically mass of the world (Bhat and Bandhu, 1994). Water pollution has been intensified by industrial and agricultural insecticides, herbicides and fungicides, which have caused aquatic eutrophication in water bodies and "Chemical Time Bomb" (Stigliani *et al*., 1988, Stigliani, 1993, Hackstra, 1991, Ali Khan and Ahmad, 1998).

Gangol Sehkari Dugdh Utpadak Sangh Ltd., Partapur, Meerut is one of the 10^{th} modern dairy industries (Pasteurization plant) of western U.P., which is located geographically 29° north & 77° 40' east and situated at 60 km north east of Delhi. Parag dairy generates 2000 to 3000 liters waste water after pasteurization of milk, cream, butter, milk cake, cheese powder etc. The dairy waste water contains caustic soda, amyl alcohol, nitric acid and sulphuric acid with high B.O.D. and acidic pH (6.5) but contains fat 20%, proteins 0.15% and 109 mg/l lactose (Rodionova *et al*., 1989 and Fang, 1990). Now-a-days, use of effluent is beneficial in agriculture because of its value as a potential and a nutrient donor. Present investigation has aroused the interest because no efforts have been made to study on environmental pollution caused by Parag dairy Partapur, Meerut (U.P.). So, systematic disposal of dairy effluent acts as "Liquid Fertilizer" into cultivable land for agro-ecological effects on crop and its impact on soil environment. Therefore, keeping above facts in view, present study was designed to evaluate the risk assessment of dairy effluent on environmental management and its effect on the soil.

MATERIAL AND METHOD

The present investigation has been conducted to observe the physico-chemical characteristics of dairy effluent of Gangol Sehkari Dugdh Utpadak Sangh Limited (Parag) Partapur, Meerut and its effect on physico-chemical properties of the soil. The experiment was conducted in college campus with three cvs. of *Brassica juncea* L. Czern & Coss. viz., Pusa Bold, Pro-Agro 4001 and T-59. The effluent was collected at 10:00 a.m. for physico-chemical examination. The untreated samples were collected from the main drain of industry before entering into the Effluent Treatment Plant (E.T.P.). The treated samples were collected from outlet of the aeration tank, where biological treatment was given (Fig 1).

Experimental Design: Sterilized and dried seeds of three cultivars of *Brassica juncea* L. viz., Pusa Bold, Pro-Agro 4001 and T-59 were sown during winter season at the college agricultural research farm in sandy loam soil. The experiment was conducted in randomized block design (RBD) with three replications. Growth parameters were observed at 30, 45, 60, 75, 90 and 105 days after sowing (DAS).

Soil Analysis: The estimation of NPK (Nitrogen, Phosphorus and Potassium) content, pH and E.C. (Electrical conductivity) of fallow (Control) soil was done before applying the dairy effluent and thereafter it was analysed at harvest condition of the crop treated with effluent. Nitrogen was estimated by modified Kjeldahl's method, phosphorus by Vanado-molybdo-phosphoric yellow color method, potassium by Flame photometer and pH by pH meter.

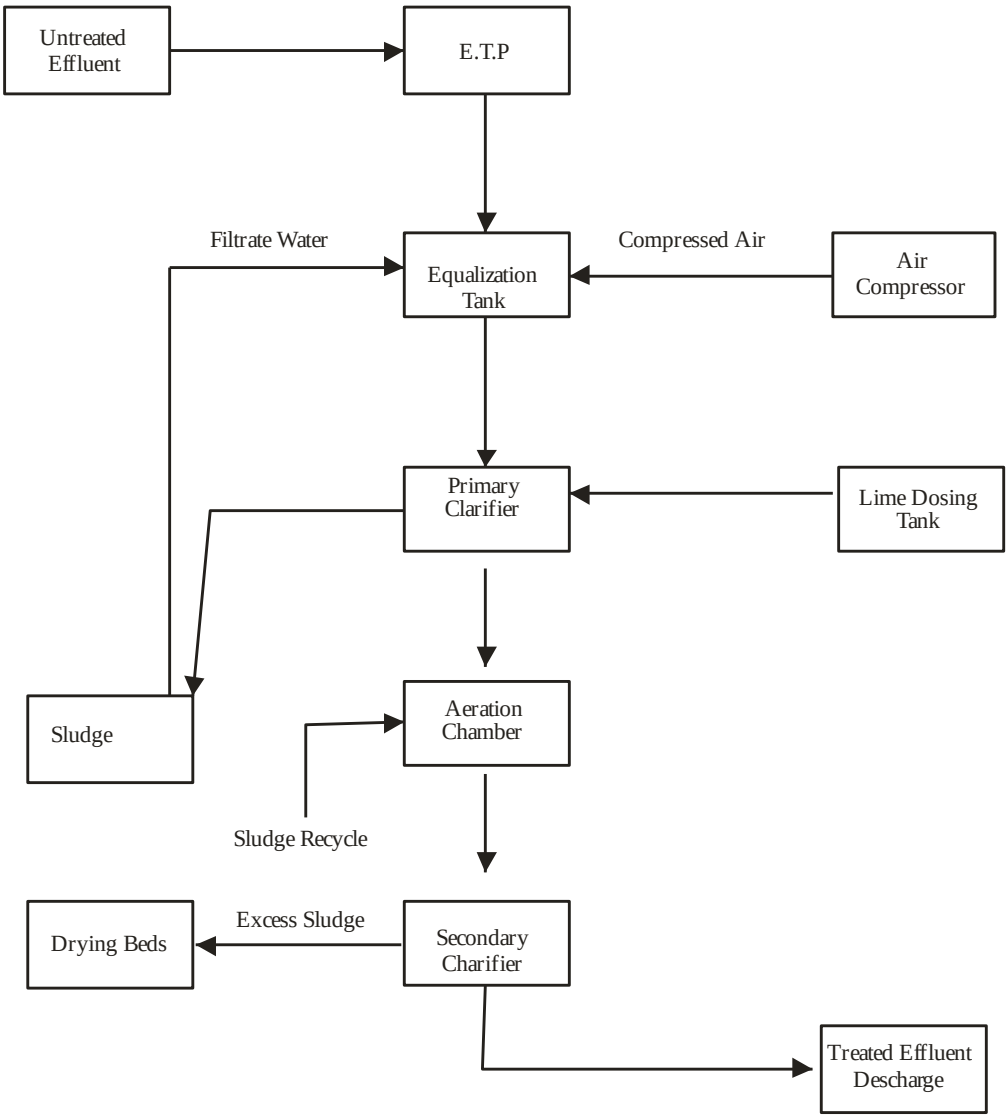

Fig 1. Flow chart of effluent treatment plant (E.T.P.).

OBSERVATION

The soil represents most important natural media for plant growth and filtration system. They can effectively decompose organic compounds, recycling nutrients and protect the environment by removing substances from percolating water. Soil has immeasurable economic importance as a substratum. The soil pH, E.C. and N.P.K. have marked effect on the growth performance and environment. In the present study data on the physico-chemical characteristics of the soil without irrigation of dairy effluent (control) and after harvesting the crop have been studied. The soil samples were collected from each plot of different cvs. of *Brassica juncea* L.

Parameters ranged from pH (7.92), E.C. (0.17 m mho/cm), N (0.40%), P_2O_5 (10.30 kg/ha) and K_2O (49.0 kg/ha) have been analysed in the fallow (control) soil (Fig 2).

Pre-sowing irrigation effect of effluent on the soil

pH: It ranges from 7.11 (25%), 7.10 (50%) & 7.09 (100%) and 7.13 (25%), 7.12 (50%) & 7.10 (100%) in untreated and treated effluent, respectively (Fig 2).

Electrical conductivity (E.C.) m mho/cm: It was observed 0.40 (25%), 0.41 (50%) & 0.42 (100%) and 0.41 (25%), 0.43 (50%) & 0.44 (100%) in untreated and treated effluent, respectively.

Nitrogen (kg/ha): It was recorded 0.51 (25%), 0.52 (50%) & 0.54 (100%) and 0.52 (25%), 0.54 (50%) & 0.56 (100%) in untreated and treated effluent, respectively (Fig 2).

Phosphorus (P_2O_5) kg/ha: It was analysed 12.30 (25%), 14.60 (50%) & 15.30 (100%) and 13.40 (25%), 15.50 (50%) & 16.60 (100%) in untreated and treated effluent, respectively.

Potassium (K_2O) kg/ha: It was found 57.0 (25%), 59.0 (50%) & 62.0 (100%) and 58.0 (25%), 60.0 (50%) & 63.0 (100%) in untreated and treated effluent, respectively (Fig 2).

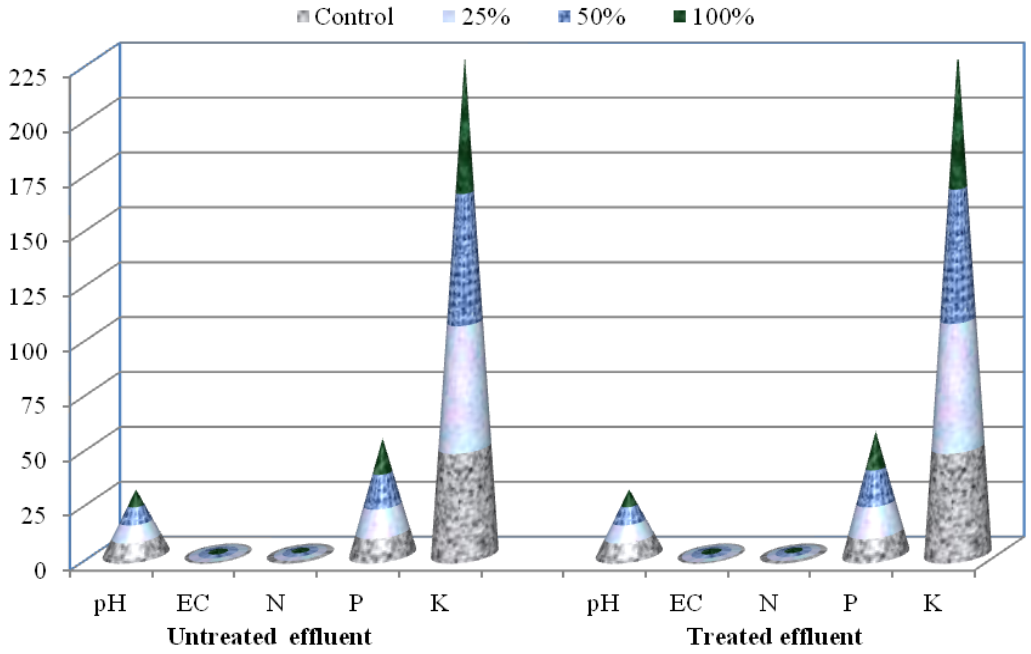

Fig 2. Pre-sowing irrigation effect of Parag dairy effluent on physico-chemical characteristics of the soil.

Soil analysis after harvesting the crop

After harvesting the crop, following parameters were observed (Table 1).

pH: It was maximum in treated soil of *Brassica juncea* cv. P.A. 4001 and minimum in the soil of cv. Pusa Bold, while the effluent concentrations decreased the pH value due to acidic nature of dairy effluent in both untreated and treated treatments.

Electrical conductivity (E.C.) m mho/cm: It was observed that effluent concentrations gradually increased the value of E.C. in all cvs. However, minimum E.C. was found in cv. Pusa Bold in comparison to cvs. P.A. 4001 & T-59.

Nitrogen, Phosphorus and Potassium (N.P.K.): It has been recorded that N.P.K. of the soil increased significantly in all the cvs. as compared to control. However, it was maximum in cv. Pusa Bold.

Table 1. Residual effect of Parag dairy effluent application on physico-chemical characteristics of the soil after harvest.

Treatments	Cultivars	pH	EC (m mho/cm)	N (%)	Phosphorus P_2O_5 (kg/ha)	Potassium K_2O (kg/ha)
Control	Pusa Bold	7.93	0.15	0.40	10.25	48.00
	P.A. 4001	7.95	0.14	0.39	10.00	47.00
	T-59	7.94	0.14	0.38	9.60	46.00
Untreated Effluent						
25%	Pusa Bold	7.18	0.41	0.45	11.40	55.00
	P.A. 4001	7.26	0.28	0.44	10.60	53.00
	T-59	7.20	0.21	0.43	10.50	50.00
50%	Pusa Bold	7.17	0.42	0.48	12.60	57.00
	P.A. 4001	7.21	0.31	0.47	11.80	55.00
	T-59	7.18	0.23	0.45	11.70	52.00
100%	Pusa Bold	7.15	0.44	0.50	14.20	59.00
	P.A. 4001	7.20	0.34	0.49	13.20	57.00
	T-59	7.16	0.25	0.48	12.10	55.00
Mean	Pusa Bold	7.16	0.42	0.47	12.73	54.66
	P.A. 4001	7.22	0.31	0.46	11.86	55.00
	T-59	7.18	0.22	0.45	11.76	52.33
Treated Effluent						
25%	Pusa Bold	7.17	0.42	0.46	11.60	57.00
	P.A. 4001	7.27	0.29	0.45	10.80	55.00

	T-59	7.19	0.22	0.44	10.75	52.00
50%	Pusa Bold	7.16	0.43	0.49	12.90	58.00
	P.A. 4001	7.20	0.32	0.48	12.00	56.00
	T-59	7.18	0.25	0.47	11.95	53.00
100%	Pusa Bold	7.14	0.46	0.52	14.40	60.00
	P.A. 4001	7.16	0.36	0.51	13.50	58.00
	T-59	7.15	0.25	0.49	13.30	57.00
Mean	Pusa Bold	7.15	0.43	0.49	12.96	58.33
	P.A. 4001	7.21	0.32	0.48	12.10	56.33
	T-59	7.17	0.23	0.46	12.00	54.33

DISCUSSION

The pH of the soil can have a dramatic effect on crop performance by influencing the solubility and availability of nutrient and non-nutrient elements. Thus, pH is an important factor in the characterization of the soil system. pH of the soil indicates the availability of mineral nutrients. Approximately, pH (6.5) of all mineral salts is sufficiently soluble to satisfy plant needs but increasing deviation to either direction certain nutrients become less soluble. Also nitrification of plant is compared below 6.0 and above 7.7 pH (Daubenmier, 1970). pH of the soil decreased with increasing the conc. of the effluent. It may be due to the acidic nature of the effluent. The industrial effluent generally affects the pH of the soil according to their own pH as has been clearly indicated by some of the studies (Ajmal *et al*., 1984; Singh and Mishra, 1987).

High value of electrical conductivity of effluent indicates preponderance of dissolved salts which leads to the increased conductivity of effluent application. The differences in the values for investigated parameters between treated and control soil indicate the probable concentration of these parameters through the effluent. The differences in the values due to treatments are significant for almost all the plant parts of both types of soil raised plant, presence of nutrients responsible for increase into available plant nutrients (Igbounamba, 1972). Vandamme and Renterghem (1981) analysed sludge and dairy effluent from its composition and agriculture fertilizers (N_2 and P_2O_5). Davis and Burgoa (1995) revealed the runoff and leaching of crop nutrients from dairy effluent into soil tithed beds and discussed decreased NO_3^- leaching and it was suggested that as effluent application rate increased the large potassium concentration in the effluent exchanged with soil calcium and magnesium.

Goel and Mandavekar (1983) have discussed the higher absorption of nitrogen from the distillery effluent irrigated soil. Sachan and Menon (1976); Arceivala (1981) concluded that 10% diluted distillery waste can effectively used for irrigation of *Cyamopsis tetragonoloba*, which will increased the vigour of plants resulting in more yield and rich in protein. Application of phosphate fertilizer has led to the accumulation of phosphorus in soil, resulting in depletion of phosphorus absorption/buffer capacity. Cadmium present as an impurity of phosphate fertilizer is applied in advently during fertilizer application, sewage sludge and contaminated manure (Stigliani, 1993). Potassium is one of the macro nutrient and acidly present in larger quantities than those of any other mineral nutrients derived from the soil with the exception of hydrogen and nitrogen. It was observed that soil pH decreased with increase dairy effluent conc. because dairy effluent is acidic. The available nitrogen, phosphate and potassium increased with increase in the conc. of dairy effluent added. Srikantha *et al*. (1998) have illustrated similar observations.

CONCLUSION

Soil pH decreased non-significantly with effluent irrigation and gradually increased after harvesting the crop in comparison to fallow (control). Nitrogen (%), phosphorus (P_2O_5 kg/ha) and potassium (K_2O kg/ha) contents of the soil increased significantly in effluent treated plots. However, it was observed that N.P.K. of the soil much increased after harvesting the crop, which improved soil fertility for integrated nutrient management system (I.N.M.S.) and agro-ecosystem.

Recommendation

Research findings recommended that effluent should be properly treated before its disposal into available land then it could be used as “liquid manure”, which is one the best practical method for the disposal of dairy effluents to eliminate pollution problems for “evergreen revolution”. Therefore, effluent treatment plant (E.T.P.) should be installed in each pasteurization plant like distilleries to check water pollution so that water bodies like holy Ganga can be saved from deterioration. Further it will not only arrange marriage between environment and economy but would have also cascading effect on national economy to curb price inflation of fertilizer. Moreover, it will be a tool to handle “Chemical Time Bomb” in soil and sediment in world scenarios.

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ETHNOVETERINARY VALUES OF SOME PLANTS USED AGAINST SNAKE BITE IN POONCH DISTRICT OF JAMMU AND KASHMIR (INDIA)

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Abstract: Poonch district of Jammu and Kashmir state possesses a rich history and culture of tribal society which have a great wisdom of traditional knowledge with regard to medicinal plants for the treatment of their livestock . Survey was conducted from January 2009 to December 2010 for the documentation of ethno veterinary plants used for snake bite particularly to cows, Buffaloes and Horse with the help of village elders, key informants and local healers which indicated that inhabitants of the valley utilize 22 species belonging to 16 genera and 12 families. The primary objective of the study was to explore the floristic diversity and valuable folk medicinal plants because the knowledge is confined to only local healers and it is important to record this knowledge for future generations which otherwise will be lost forever. Family name, botanical name with local name in bract, parts used, method of preparation and mode of use is presented here.

Keywords: Against snake bite, Tradional knowledge

INTRODUCTION

The documentation and analysis of traditional knowledge about the locally used resources have been an integral part of botanical research and have resulted in a rich body of knowledge about the locally used resources (Prance 2005). Poonch district of jammu and Kashmir state supports a wide variety of floristic diversity and a large number of livestock population which play a vital role in the economy of tribal and rural people. District is one of the most important trible populated area of the state who inhababit from Tehsil Mendher to upper Pir Panjal range. Gujjars, Bakerwals and Paharis are the major tribes of the district. Considerable work on ethno medico botany as well as on floristic diversity has been done from different states of India as well as from different countries by different workers like Hooker (1872-1897) worked on flora of British india,Duthie(1893-94) visited the Kashmir and prepared a report on botanical tour of Kashmir,Nasir and Ali (1970) worked on flora of west Pakistan, Koul *et al*. (1989)worked on ethno botany of Bani Basoli region of J&K state, Pangtey (1989) has worked on ethno botanical notes on Bhutia tribes of Kumaon Himalya, Aswal; (1996) workd on conservation of ethno medicinal plants diversity of Garwal Himalya. Koul (1997) worked on Medicinal Plants of Kashmir & Ladakh, temperate and cold arid himalya. Lan and Brown (1998) worked on ethno veterinary medicine used for ruminant in Trinadad and Tobago, Reddy *et al.* (1998) worked on plants used in ethno veterinary practices in Wrangal District Andhra Predesh. Gour (1999) worked on Flora of District Garwal North West Himalya with ethno botanical note, Jain similarly (1999)worked on ethno veterinary plants of India. Reddy and Raju (1999) studied on plants in ethno veterinary practices in, *Anantpur* district of Andhra predesh, Raju and Raju similarly(1999)worked on plants used in ethnovetrinary practices in Anantpur district Andhra Predesh , Reddy *et al.,* worked on ethno veterinary plants used by Gonds of Karimnager District Andhra Pradesh. Raju (2001) also studied ethno veterinary medicine in Andhra Pradesh whereas Tomar and Singh (2006) studied ethno therapeutics of some medicinal plants from Khatauli Block of Muzafarnagar district. Raju 2007 studied on ethnoveterinary medicine in Andhra predesh. Chourasia *et al.* (2007) also studied the ethno botany and Plants of Trans Himalya. Although enough from other districts of Jammu and Kashmir state and different states of India but little attention has been paid on poonch district of Jammu and Kashmir in relation to ethno veterinary plants used for snake bite.

Study area : Poonch district of jammu and Kashmir state situated in North west Himalya lies between 73˚ 30' to 74˚ 35' east longitude and 33˚ 35' to 34˚ 10' north latitude in the southwest of Srinager .It lies at about 100 m above sea level on the northern bank of poonch river and connects Jammu the winter capital of the state via Bhimber gali and Rajouri and Srinager the summer capital of the state via Pir gali and Shopian It enjoyes an average subtropical climate with heavey rainfall in summer and moderate winter Pir panjal is the barier between poonch and Kashmir and also for the monsoon for crossing over to Kashmir valley. Jammian wali gali, Nur pur gali and Pir gali are the most important tracks used by Gujjers and Bakerwals for crossing over to Kashmir valley along with their livestock. Pir Panjal is the barier between poonch and Kashmir and also for the monsoon for crossing over to Kashmir valley.The track from Buffliaz to Pir gali has also been used by Mughal emperors to enjoy the beauty of Noorichamb waterfall and high altitude alpine meadows. The mountain and peaks of Pir Panjal rang are of considerable height ranging between 3000 m to 5000 m and above. Adminstratively the district is comprised of 6 C D Blocks 4 Tehsils and 115 Panchyets with a total area of 1674 sq Km.

Geologically most parts of the district are made up of shale with traces of bauxite, graphite,lignite etc. The rocks are little over 1 million year old of the Pleistocene age (Wadia 1939).The area has experienced volcanic eruptions followed by glaciations in the geological past, which have left lot of morainic material. The district is drained by poonch river of Tehsil Havelli and Banloi river of Tehsil Mendher.The PH of soil ranges between 5.5 to 7.8. Maiz, Paddy and Wheat are the major crops of the same area.

METHODOLY

Survey was conducted in different villages and panchayets of the poonch district of jammu and Kashmir during the year 2009 and 2010. It comprised particularly in Pathanatir, Kalaban ,Harni, Dharana, Gursai, Snai, Chandimar, Khaneter, Sathra, Kehnu and Bufliaz and also in upper alpine meadows of chata pani, Ghas, jamian wali gali,and Nurpur gali. Documentatio regarding ethno veterinary plants for snake bite was made by direct interviewing of medicine mem, women and grazers of long experience. During the field visit we practiced group interviews whenever possible, but conversation with only one informant was a common practice, so the results were authenticated by cross intervening of three to four persons. The plant material was collected freshly accompanying the knowledgeable people from respective villages and standard herbarium technique (Jain & Rao ,1977)was used for preservation. The voucher specimens were identified with the help of standard floras and by matching with the herbarium of Indian institute of integrative medicine Jammu. The Standard methodology regarding the documentation was followed as suggested by Shultes(1960),Jain (1981,1987) and Ford(1978).The identified herbarium specimens were deposited in the department of botany Kisan (P.G.) College simbhoali for further use.

RESULT AND DISCUSSION

The author collected about 60 taxa of the plant from different localities of the district. Out of these 21 are used against snake bite belonging to 16 genera 22 species and 12 families. The local use of plants as a cure is common particularly in those areas which have little or no access to modern health care facilities. This traditional knowledge of medicinal plants is fast disappearing due to scarcity of written documents and relatively low income in these traditions

The folk healers prepare a certain dose of medicines taking into consideration the physical activity of the victim after bite, which depends upon the severity of the envenoming species, length of the snake and the amount inoculated. The severity of bites from different species is sometimes is increased due to primary bacterial infection present in the oral cavity of the snake which means that antibiotic treatment is sometimes necessary. Furthermore the effectiveness of the herbal remedy also depends upon the proper time of collection and preservation. The dose depicted here is used as a first aid in rural and far flung areas and is given above the age of two years and may be increased or decreased depending upon the physical condition of the victim. About 20 to 25 gram dried tuber of the *aresaema* spp is also given daily by wrapping in butter if swelling occurs for a long time. Special care is taken while giving *Aresaema* spp and *Aconitum voilacium* so that they do not touch the tongue and pharynx because it may cause swelling in the digestive tract. Although the tribal and rural are not adverse in accepting the modern medicine but about 80 to 90 percent of them still depend upon the surrounding vegetation to cure the victim of snake bite. For the systematic enumeration the plants are listed alphabetically with their botanical name, local name in bract, family, parts used, method of preparation and mode of use are describing in detail.

Table 1. Enumeration of some plants used against snake bite

S. No.	Botanical name (Local Name)	Family	Parts used	Method of preparation and mode of use
	Achillea millefolium Linn. (Chau, Pehlkach)	Asteraceae	Root	About 50 – 60 gram of root is crushed with stones and given orally to cows and buffaloes.
	Aconitum heterophyllum Wall ex Royeli (Patrees)	Ranunculaceae	Root	About 50- 80 gram powdered or crushed root is given to buffaloes, ox and horse.
	Aconitum voilacium Jacquim ex Stapf (Mori)	Ranunculaceae	Root	Paste of the root is applied externally. About 4 to 5 grame may also be given internally by wrapping in wet wheat flour or butter but taking into consideration the physical activity of the victim after bite.
	Acorus calamus Linn. (Pyonzkartal, Bach)	Araceae	Rhizome	About 100 to 150 gram of crushed rhizome is given to horse orally.

	Aresaema flavum forsk (Sap ni mak)	Araceae	Tuber	Tuber of the plant freshly collected during the month of August to September is crushed with stones and about 100 to 150 gram may be given orally by wrapping in wet wheat flour or butter. 30 to 35 gram dried and powdered tuber is also given by wrapping in wheat flour.
	Aresaema jacquimontii Blume(Sap ni mak)	Araceae	Tuber	Freshly collected tuber during the month of August to September is crushed with stones and about 100-150 gram is given orally by wrapping it in wet wheat flour. 30 to 35 gram powdered tuber is also given orally by wrapping in butter.
	Areaema propinquum Schot (Sap ni mak)	Araceae	Seeds	About 100 to 150 gram seeds of the plant are also given orally by crushing with stones and warping in wet wheat flour. Paste of the seeds is also applied externally. Antidote to all the species of Aresaema is butter and milk.
	Asplenium dalhouseae Hook (Sap ni jari, Alaf wali jari)	Aspleniaceae	Whole plant	200 to 300 gram whole plant is crushed with stones and given internally followed by 20 to 40 gram Gol Mirch (*Pipper nigrum*) and desi ghee
	Alium cepa Linn. (Payaz)	Lilliaceae	Bulb	Paste of the bulb is applied externally and about hundred to two hundred gram is given internally followed by 10 to 15 gram Gol Mirch.
	Alium sativa Linn. (Thoom)	Lilliaceae	Bulb	50-60 gram bulb is made into paste and is given orally along with butter.
	Barleria cristata Linn. (Sap ni jari)	Acanthaceae	Whole plant	About 200 to 300 hundred gram whole plant is made into past and given orally.
	Bupleurum falcatum Linn. (Peeley phul wali jari,nagdun)	Apiaceae	Whole plant	About 100 to 200 gram whole plant is grinded with stones and isgiven orally to cow's buffaloes and horse.
	Delphinium denudatum Wall (Sap ni jari)	Ranunculaceae	Root	10 to 20 gram powdered root is given orally .past of root is also applied externally.
	Dioscorea bulbifera Linn. (Chachla ganda, Kala ganda ,Kithi ganda)	Dioscoreaceae	Tuber	About 200 to 300 gram tuber is given orally by mixing in 10 to 15 gram Gol Mirch (*Pipper nigrum*).
	Gloriosa superba Linn. (Sap ki jari)	Lilliaceae	Root	About twenty gram root is grounded and given orally.
	Micromeria biflora Buchanan Hamilton ex D Don, Benth (Chickni)	Lamiaceae	Whole plant	200 to 300 gram whole plant is grounded and given orally.
	Podophyllum hexandrum Royle (Bankhakri wanwangun)	Podophyllaceae	Rhizome	Past of rhizome is applied externally and about 30 to 40 gram is given orally.
	Primula denticulate Smith(Landanposh)	Primulaceae	Whole plant	150 to 200 gram plant is crushed with stones and is given orally.

	Primula macrophylla Don (Ladanposh)	Primulaceae	Whole plant	About 200 gram whole plant is grinded and given orally.
	Sorghum halepense Linn. (Baru)	Poaceae	Root	About 100 to 150 gram root is grinded and given orally.
	Thymus linearis Benth ex Benth (Chickni)	Lamiaceae	Whole plant	100 to 150 gram whole plants is given orally.
	Vitex negunda Linn. (Bana)	Verbenaceae	Leaves	Leaves of the plant are made into paste and 100 to 200 gram is given orally.

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Report

BIOPROSPECTING ANTICARCINOGENIC POTENTIAL OF PLANTS IN RAJASTHAN, INDIA

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Abstract: Purpose of this communication is to report on the present status of anticarcinogenic plants of Rajasthan based on folk lores, ethnobotanical , pharmacological and biochemical studies meant for more fruitful and directed future studies and projects. An up to date account of antioxidants, antimutagens, detoxicants anti-inflammatory and antiproliferative. antimetasis, antiangiogenic plants has been given.

Keywords: Bioprospecting, Anticarcinogenic, Antioxidant plants, Rajasthan

INTRODUCTION

Uncontrolled growth of body's own tissues and organs due to genotoxic and epigenetic carcinogens is called cancer, a leading cause of mortality worldwide. In India, every year about 65,00000 new cancer cases are diagnosed resulting in about 58,0000 cancer related death of which, oral and lung cancer in men and breast and cervical cancer in women predominate. These are basically caused by oxidative stresses caused by modifiable and unmodifiable factors. Dominant carcinogens are Nitroso foods, contaminated aflatotoxigenic foods, infections, occupational pollutants, poor diet, obesity, tobacco chewing & smoking, alcoholism, radiation exposure and drugs (Singh *et al.*, 2009).

A variety of synthetic and natural anticancer agents with various degree of chemopreventive values have been identified to combat cancer but treatment of cancer by synthetic drugs is generally not preferred because of their high cost, range of side effects, toxicity, drug resistance and large number of consequent dropouts. Hence, bioprospecting novel anticancer agents from traditional medicinal and dietary plants and their products is the oldest and most effective strategies that date back to Chinese and Indian Ayurvedic medicines (Sharma & Kumar, 2000)

Recently, there have been exponential increases in the quantum of studies investigating cancer chemoprevention by plants especially in poor countries. These anticarcinogenic investigations use several complementary and overlapping mechanisms, such as, inhibition of activation of procarcinogens, enhancement of detoxification pathways, antioxidants, antimutagens and alteration of cellular signal pathways (Singh & Praceta, 2012). In Rajasthan, majority of studies concern Antioxidant plants apart from Ethnobotanical studies showing such activities. Antioxidant system tends to reduce Free Radical formation, balences oxidative stress implicated in tumour initiation, Cell proliferation and differentiation besides apoptotic induction by diverse stimullii (Goyal & Sharma, 2009) hence are justified for bioprospecting anticancer herbal drugs.

Ethenic Tribes, Ethnomedical and Ayurvedic Studies in Rajasthan having Anticarcinogenic potential: Asurs, Bhils, Bhungi, Cherches, Garos, Gonds, Hoes, Jaintians, karses, Khases, Konds, Lodhas, Mahles, Mudras, Marias, Mishrees, Rotha, Soras, Lohars, Santals and Shonpers are main tribes of Rajasthan and are distributed In Dausa, Jhalwar, Udaipur, Chittod, Jodhpur, Jaipur, Banswara, Ajmer, Mount Abu and Alwar areas. Because of dwindling ethnicity and likely folk lores and traditional knowledge, several investigators have, therefore, explored Ethnobo-tanical/Ethnomedicinal wealth of Rajasthan used by Bhopas to treat various ailments including tumours. Sood *et al.* (2005) have prepared a compendium on Ethnic plants of India used in cancer cure. Garg *et al*. (1980) made survey for Alkaloids in Rajasthan Desert plants. Ethnobotanical studies on fern allies of Rajasthan were undertaken by Sharma and Vyas (1985) who found several species of *Selaginella, Equisetum, Marsilia, Adiantum, Pteris,Angiopteris and Blechnum* to possess anticancer properties. Dhanukar *et. al* (2000) reported on the pharmacology of medicinal plants and natural products but did not cover much of the anticarcinogenic plants.However, anti-inflammatory plants like *Azadirachta indica, Butea monosperma, Calotropis procera, Gymnema sylvestre, Gmelina asiatica, Ocimum sanctum* and *Pongamia pinnata* were reported by them.This was followed by an overview on medicinal Pteridophytes by Dixit and Singh (2004). Antitumour properties in Pteridophytes have also been described by Singh and Pracheta (2012). Sharma and Kumar (2001) searched for anticancer drugs in traditional medicines. Chaudhary and Swarnkar (2001) reported on antioxidant activity of phenolic and flavonoid compounds in some medicinal plants of India. Anti-inflammatory and antitumour properties were reported in ethnomedical plants like *Bombax ceiba, Citrus medica, Cleome gynandra, Commiphora wightii, Ipomoea muricata, lomonis acidissima* and *Salvodora oleoides*, medicoethnobotany of Dausa Distt. was explored by Sharma and Trivedi (2004) who reported several antioxidantive plants,such as *Aegle marmelos, Achyranthes aspera, Aloe vera, Azadirachta indica,*

Bacopa, monnoeri, Barleria prionitis, Butea monosperma, Cassia angustifolia, Cassia fistula, Calotropis procera, Compmiphora wightii,Curcuma longa, Gymnema sylvestre, Picorrhiza kurroa, Tinospora cordifolia, Withania somnifera, Euphorbia hirta , E. neriifolia, Lawsonia inermis, Moringa oleifera, Pterocarpus sp, Tamirindus indica, Vitax negundo, Acacia nilotica, A. catechu, Dalberjia sissoo Phyllanthus emblica and Tecomella undulata but Local herbalists were unaware of anticarcinogenic potential in them albeit searching for anticancer drugs in traditional medicines was undertaken by Sharma and Ashwani Kumar (2001). Sharma (2004) studied ethnomedic-oreligious plants of Hadoti plateu of Rajasthan and reported *Acacia nilotica, Aegle marmelos, Azadirachta indica, Butea monosperma, Calotropis gigantica, Calotropis procera, Ficus religiosa, Gloriosa superba, Solanum surattense, Syzium cumini* etc. Chaudhary, Singh and Pillai (2008) also made ethnobotanical survey of Banswara Distt of Rajasthan and reported anticancer properties in *Leea macrophylla* and *Sauromatus venosum*. Suresh Kumari *et al.* (2008) also investigated antioxidant activity in some selected Indian medicinal plants. Besides reporting on anticancer *Albizzia amores, Achyranthes aspera, Cassia fistula, Curcuma longa* and *Gloriosa superba*., Jain, Jain and Singh (2009) made ethnobotanical survey of medicinal plants of Sariska and Siliserh region from Alwar Distt of Rajasthan including *Coccinia cordifolia, Catharenthes roseus, Sida rhombifolia, Salvadora oleoides, Aegle marmelos, Achyranthes aspera, Asparagus racemosus, Berleria priontis, Boerhaavia diffusa, Corchorus aestuansGrewia tenax, Emblica officinalis, and Terminalia arjuna* which are now known to be anticarcinogenic.

Anticarcinogenic Studies: Recently, there has been exponential increase in the number of studies investigating cancer chemoprevention in plants. These cancer preventing anticarcinogenic studies use several overlapping and complementary mechanisms including 1.initiation of activation of procarcinogens 2. Antimutagens 3. stimulation of Detoxification 4.Antioxidants 5. Alteration of cellular signal pathways. As Antioxidant system tends to reduce free radical formation, balances oxidative stress implicated in tumour initiation,proliferation, differentiation as well as Apoptosis induction by diverse stimuli (Goyal and Sharma,2009), hence present attempt of bioprospecting Antioxidant plants of Rajasthan in Anticarcinogenesis is justified: Singlu,Jain & Jain (2009) observed Antioxidant activity of some medicinally important Arid Zone plants,such as, *Aerva tomentosa, Helitropium sp, lepidagathes trinervis, Mimosa hamata, Molligo nnudicaudis, Trianthema decandra, Tribilus terristres, Verbesinea enchiides,polycarpea corymbosa, Potulaca pilosa and Gisekia plannacioides.* Mandal et al (2009) and Panchawat *et al*. (2010) reviewed our information on Herbal Antioxidants .Chaudhary, Saroha and Swarnkar (2011) observed Antioxidant activity in *Acacia nilotica, Delbergia sissoo, Delonix regia, Catharenthes roseus, Mentha arvensis, Ocimum basilicum, and Tinospora cordifolia* etc while Kumar *et al*. (2011) compared Antioxidant activity in hepatoprotective plants . An overview on potent herbal Antioxidant drugs was presented by Rajwar *et al*. (2011).

Documented Antioxidant Plants of Rajasthan: Due to the presence of Polysaccharides, Phenolics, Alkaloids, Flavonoids, Glucosides and Caronetoids and plants are known to possess antioxidantive properties, Many such plants have been reported from Rajasthan as mentioned below: 1.*Aerva tomentosa* (Sethi & Sharma, 2011) 2. *Asparagus racemosus* (Patro & Bhatnagar, 2005; Viral,2005) 3. *Acacia nilotica* (Meena *et al.,* 2006) 4. *A. arabica* (Gupta & Bokadia, 1975) 5. *Azadirachta indica* (Koul *et al.*, 2006) 6. *Amaranthus paniculata* (Bhatia & Jain, 2002) 7. *A. gangeticus* (Verma, Sisodia and Bhatia, 2002) 8 *Andrographis paniculata* (Handa & Sharma, 1990; Tanwer *et al.*, 2010) 9. *Achyranthes aspera* (Srivastava *et al.,* 2011) 10. *Alstonia scholaris* (Swafiya *et al.*, 2010) 11. *Acacia catechu* (Syed & Asad, 2009; Karwani, *et al.*, 2011) 12. *Aegle marmelos* (Rajan *et al*., 2011) 13. *Aloe ver*a (Panche, Kumar & Kumar, 1998; Saritha *et al.,* 2010) 14. *Boerhaavia diffusa* (Chakraborti & Handa, 1989) 15. *Bougainvillea glabra* (Gupta & Rathore, 2011) 16. *Barleria priontis* (Singh *et al.*, 2005) 17. *Balanitis roxbughii* (Singh *et al*., 2009) 19. B. aegyptica (Vijay & Vijayvergis, 2010) 20. *Bauhinea variegata & Leucas aspera* (Bhatia *et al*., 2011; Gupta, Bhattacharya, Sharma & Nareg, 2010; Gupta *et al.*, 2011) 21. *Butea monosperma* (Sharma & Deshwal, 2011) 22.*Cissus quadrangulaenes* (Vijay *et al.,* 2010) 23. *Corchores aestuares* (Patel, 2011) 24 *Chlorophytum borivilliarum* (Kumar *et al.*, 2010) 25. *Cyperus rotundus* (Yazdenparst and Ardestam, 2008) 26. *Cleome viscosa* (Gupta & Dixit, 2009) 27. *Euphorbia neriifolia* (Sharma, Pracheta & Singh, 2011; Sharma, Pracheta, Paliwal, Singh, & Sharma, 2011) 27. *Garuga pinnata* (Kathod *et al.*, 2010) 28. *Glycosmis pentaphylla* (Gupta *et al.*, 2011) 29. *Grewia asiatica* (Ahaskar *et al*., 2007) 30. *Gisekia plannaceioides* (Singh, Jain & Jain, 2009) 31 *Moringa oleifera* (Sharma *et al.*, 2011) 32 *Momordica charantia* (Chaudhary *et al*., 2009) 33 *Peltophorum pterocarpus* (Jain *et al.*, 2011) 34 *Prosopis cinerariae* (sharma *et al.,* 2010; Dharni *et al.*,2011) 35.*Portulaca oleracea/pilosa* (Sanja *et al*.,2009; Dejas *et al*., 2002) 36 *Polycarpa corymbosa* (Hukkeri and Kenganora, 2007) 37. *Phyllanthes niruri, P. emblica* etc. (Harish & Shivandappa, 2005; Kumaran & Karuna kumar, 2007) 38. *Rubus ellipticus* (Sharma & Kumar, 2011) 39. *Pongamia pinnata* (Sangwan *et al*., 2011) 40. *Mentha piperita* (Samarath, Goyal & Kumar, 2001) 41. *Terminalia*

arjuna (Singh *et al.*, 2011) 42. *Tecomella undulata* (Singh & Gupta, 2011) 43. *Tinospora cordifolia* (Sharma *et al.*, 2011) 44. *Triumfetta pilosa* (Ramakrishna *et al*., 2011) 44. *Tribulus* terrestris (Pandey, Bhavani & Saini, 2007) 45. *Thuja occidentalis* (Dubey & Batre, 2009) 46. *Sympploca racemosa* (Vimal, 2010) 47. *Cassia sophera* (Nagore *et al.*, 2010) 48. *Panex ginseng* (Verma, Jahan, Kim & Goyal, 2011) 49.*Becopa monnieri, Terminalia chebulae* & *Picrirrhiza kurroa* (Kumar, Singh & Singh, 2008) 50. *Withania somnifera* (Devi, 1996; Sharma *et al.*, 2011) 51. *Calotropis procera*/ *Calotropis gigantia* (Suresh & Chauhan, 1992; Joshi *et al.*, 2010) 52. *Salvadora oleroid*es (Yadav *et al*., 2008; Jain, Jain & Singh, 2009) 53. *Spinacea oleracea* (Bhati & Jain, 2004) 54, *Glycyrrhiza glabra* (Dayanan *et al.*, 2010). A delving back in ethnomedical literature reveals Antioxidant plants,such as, *Leea macrophylla, Sauromantusa venosum, Bombax ceiba* (Gandhare *et al.*, 2010), *Cassia fistula, Curcuma longa, Calotropis procera, Dalbergia sissoo, Delonix regia, Cyperus rotundus* (Yazdenparst and Ardestam, 2008), *Rosmarinus officinalis, Nerium indicum, Murraya koinighii, Ocimum basilicum, Tamarindus indica, Solanum surattense, Trigonella foenum graceum* ,*Sygium cumini* and *Zizyphus mauritiana* are also found in Rajasthan. (Chaudhary, Singh & Pillai, 2008; Jain, Jain & Singh, 2009; Chaudhary, Saroha & Swarnkar, 2011). Singh, Jain & Jain (2009) also reported *Helitropium marifolium, Lepidagathes trinervis*, *Mollugo nudicaulis, triantherma decandra*, *Verbesiana enchiides,*, and *Portulaca pilosa* from Rajasthan.

Bioactive Phytoconstituents of some Antioxidative plants: *Acacia nilotica* contains tannins, ellagic acid, stearic acid, carotene, ascorbic acid and selenium; *Acacia catechu*-catechin, tannic acid, quercetin; *Asparagus racemosus*-saponin, flavonoid; *Aloe vera*-emodin, barbalein, bradykininose, anthraquinones; *Alstonia scholaris*- alkaloids, saponin, triterpene, xanthine oxidase, SOD; *Azadirachta indica*-azadirachtin, gedernin; *Amaranthus paniculata*-carotenoids and vitamin C; *Achyranthes aspera*-Achyranthane, emblicin; *Andrographis paniculata*- andrographides; *Boerhaavia diffusa*- alkaloids, sterols, isofuroxanthone; *Chlorophytum bosivilianum*- flavonoids, tannins, Phenolic compounds; *Calotropis procera*- flavonoids glucoside alkaloids; *Curcuma longa*- curcumin; *Glycirrhiza glabra*-Glycirrhizin; *Corchorus aestuaris*/ *Corchorus olitorius*-Phenolics, glucisinoids; *Glycosmis pentaphylla*-flavonoids, alkaloids like naphthaquinin, acridone, avicenol; *Heliotropium indica*-Indicene N-oxide; *Coriandrum sativum*-Anethol; Mentha arvensis-L menthol, *Moringa oleifera*- Saponin, Glycosides, Flavonoids, proanthocyanidins, beta sterol; *Phyllanthus*- quercetin, flavonoids, Ascorbic acid; *Spinacea oleracea*- Ascorbic acid, beta carotene, lutein, zeaxanthin; *Euphorbia neriifolia*-Sapongin, triterpene, sterol; *Ocimum* sanctum-Orientin, vicinin; *Syzium cumini*-Ellagic acid; *Solanum surratense*-trans-squlere-9, 12, 15-octade calrieoic acid; *Salvadora*-Anthraquinone; *Tecomella undulata*-Beta sterol, COX enzymes; Leucas aspera- Leucasin; *Triumfetta pilosa*-Iridoid alkaloids, Coumarins, flavonoids, leucoanthocyanin; *Withania somnifera*-withanolids.

Antitumour anticarcinogenic plants and mechanisms:

Hepatoprotective Antioxidants: These include *Becopa monnerii, Picrorrhiza* kurroa, *Glycyrrhiza glabra, Azadirachta indica, Tephrosia purpurea, Terminalia arjuna, Tinospora cordifolia, Swertia chirata, Phyllanthus amarus,* Justicia *gendarussa Aloe vera* (Kumar *et al.*, 2011). Bhatia and Jain (2004) have reviewed information on this topic.

Antimutagenic Plants: *Acacia nilotica, Coriandrum sativum, Chrorophytum* sp. etc

Radioprotective Plants: *Panex ginseng* (Pande *et al.*, 1980 *Aloe vera* (Pande *et al.*, 1998) *Amaranthes gangeticum* (Verma, Sisodia and Bhatia,2002) *Mentha piperita, Ocimum sanctum, Amaranthes paniculata, Spinacea oleracea* etc. (Saxena, 1997; Jain, 2002 Bhatia and Jain, 2004) and Herbal preparations like Liv 52 (Constituents- *Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achilla millefolium, Tamarix gallica)*. Goyal (2004) has reviewed the subject adequately.

Anti-inflammatory plants: *Achyranthes aspera, Barleria prionitis, Azadirachta indica, Boerhaavia diffusa, Semecarpes anacardium, Aloe vera, Curcuma longa, Aegle marmelos, Tinospora cordifolia, Euphorbia nerii folia, Cassia sophera, Syzium cumini& Corchorus aestuans.*

Antiproliferative plants*: Andrographis paniculata, Panex ginseng, Garcinea sp. Gymnostemma pentaphylla, Coriandrum sativum,Butea monosperma* (Pathak *et al.*, 2011).

Plants with Antimetasis activity: eg.*Trigonella foenum graceum*.

Plants with Antimigration activity: e,g, *Euphorbia nerifolia*.

Antiangiogenic Plants; *Bombax ceiba, Delonix regia, Calortropis procera*

Plants with cytotoxic activity: *Glycyrrhiza glabra, Euphorbia neriifolia, Cyperus rotundus,Glycosmis pentaphylla.*

Plants that are Immunomodulatory: *Acacia catechu,Euphorbia spp.*

Plants that Cause Apoptosis Induction: *Tribulus terrestris, Cyperus rotundus*

Plant-derived Anticancer Drugs: 1.Bengaptin (*Balanitus aegyptica*) 2.Vincristine,Vinblastin (*Catheranthes roseus*)

Most extensively investigated Anticarcinogenic plants of Rajasthan: These are *Euphorbia neriifolia,*

Moringa oleifera and Withania somnifera and are discussed below:

Euphorbia neriifolia: Rao and Prasad (1995) purified and partially characterized a lectin from the latex of this plant. Rasik et al (1996) observed wound healing properties in the latex. Phytopharmacological review of *E. neriifolia* was undertaken by Bigoniya and Rana (2005; 2008;2010). They observed Radioprotective, Hepatotoic, Antitumour and cytotoxic properties due to saponin (2009). Gaur et al (2009) observed anti-inflammatory and antianalgestic activity. Pal, Sahu and Patnaik (2009), Sharma, Janmeda and Singh (2011) and Ahmad *et al.* (2011) observed hepatoprotective and and anticarcinogenic antioxidant activity of alcohol extract of aerial parts *of E, neriifolia*. Preliminary phytochemical screening and in vitro antioxidant potential of hydroalcoholic extract of this plant was also carried out by Pracheta *et al.* (2011) and found Alkaloids,Flavonoids and terpenoids as important constituents. In the same year, Pracheta, Sharma, Paliwal and Sharma reported on in vitro free radical scavenging and antioxidant potential of ethanolic extract of *E.neriifolia*. Pracheta *et al.* (2011) observed chemopreventive effect of hydroethanolic extract of the plant leaves against DENA- induced Renal carcinogenesis. Sharma, Pracheta, Paliwal, Singh and Sharma (2011) investigated anticarcinogenic potential of *E.neriifolia* leaves against N-Nitrosodiethylamine induced nephrotoxicity in mice. Pracheta et al (2011) also observed chemopreventive activity of hydroalcoholic extract of *E.neriifolia* leaves against DENA-induced Liver Carcinogenesis. Antitumour, antistimulatory and immunomodulatory properties of E.neriifolia appear due to Sapogenin,lectin polysaccharide ingenase. Anticarcinogenic potential in other species of *Euphorbia*,such as, *E,kansui*, *E. tirucalli*, *E.hirta*, *E.fischeriana*, *E.pephes*, *E.drumondii*, *E.petiollta*, *E. hebecurpa,E. microciaidi* has also been reported elsewhere (Chen, 2000;Patil and Magdum,2011)

Moringa oleifera: Horse Radish tree, *Moringa oleifera* has also extensively been studied with respect to its importance. A review on the commercial importance of this multipurpose tree was written by Paliwal, Sharma and Pracheta (2011). With respect to anticarcinogenic antioxidant activities , the chemomodulatory effect of *M. oleifera* leaves on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillanagenesis in mice was undertaken by Bharati, Tabassam and Azad (2003). Sharma, Paliwal, Pracheta and Sharma (2011) investigated phytochemical and antioxidant activities of the ethanolic extract of the pods of the plant in question. Paliwal, sharma and Pracheta (2011) free radical scavenging activity of aquous and hydroethanolic extract of *Moringa oleifera* pods. In the same year, Paliwal, Sharma, Pracheta and Sharma (2011) reported on hepatoprotective and antioxidant potensial of pods of this plant against DMBA-induced hepatocarcinogenesis in male mice. Paliwal *et al.* (2011) also investigated anti-nephrotoxic effect of administration of *Moringa oleifera* in amelioration of DMBA-induced carcinogenesis in Swiss Albino mice.

Withania somnifera: Bhattacharya, Satyan, Kalkunta and Shibnath (1997) observed antioxidant activity of glycowithanolides of this plant after Umadevi (1996) suggested that Ashwagandha may be a potential plant source of a promising druf for cancer chemotherapy and radiosensitisation. Prakash, Gupta and Dinda (2002) observed that withania somnifera root extract protected DMBA-induced Squamous cell carcinoma of skin in Albino mice. Sharma, Sharma, Pracheta and Paliwal (2011) reported that *Withania somnifera* is a rejuvenating Ayurvedic m edicinal herb that is useful in the treatment of many human ailments. Sharma, Sharma, Pracheta ,Paliwal and Sharma (2011) in particular observed therapeutic efficiency of W. somnifera root extract in the regulation of Lead nitrate Nephrotoxicity in Albino mice. Sharma, Sharma, Pracheta and Sharma (2011) also reported that ethanolic root extract of the plant affects Neurological parameters in mice subjected to lead nitrate. Earlier, Patro and Bhatnagar (2005) reported antioxidant effects of Ashwagandha.

Some other Anticarcinogenic plants of the State: 1. *Rosmarinus officinalis*:- Radioprotective (Sancheti and Goyal,) 2.*Bombax ceiba*: Antioxidantive, Hepatoprotective, and Antiangiogenic (Gandhare et al,2010) 3. *Butea monosperma*:- Anti-inflammatory, Hepatoprotective, radical scavenging and Chemopreventive (Sharma and Deshwal,2011) 4.*Prosopis cineraria*:- Bark is Antioxidant,Lepidemic (Sharma et al,2010) 5.*Glycosmis pentaphylla*:-Antioxidative and Cytotoxic (Gupta *et al*., 2011) 6. *Triumfetta pilosa*:- Anti-inflammatory, immunomodulatory and anticancer 7.*Aloe vera:*- Anti-inflammatory, antimutagenic, antileukemic, radioprotective, cytotoxic, antitussive and anticarcinogenic 8. *Polycarpae corymbosa*:-Antioxidative 9. *Cyperus rotundus:*-Antioxidative, Cytotoxic and Apoptotic 10 *Calotopis gigantia*:- Antioxidative (Joshi *et al.*, 2010) 11.*Cleome Vesica*:- Hepatoprotective.

ImportantDissertations and Undergoing Projects : A Saxena (1997) got his Ph.D on (Enterohepatic response against the combined effect of mercury and radiation in mice and its modification by Liv52; K Marda (1999) was awarded Ph.D on Investigation on the possible radioprotective effect of beta carotene on mice testis while M Jain (2002) got his degree on Evaluation of antioxidant efficacy of certain plants extract: A study on mice liver. These works were carried out at Rajasthan University,Jaipur.

Projects: 1.Dr. Sreemoylee Chatterjee of International College for Girls,Jaipur is working on Study of antimutagenic activity of Fennel and

Ajwain against sodium azide induced mutagenesis 2.Dr. Hemant Lal Jadhav of Jodhpur is working on Antioxidant profile of some Medicinal plants of Rajasthan 3.Dr. Prachi Tyagi of BITS, Pilani is working on Studies of antioxidant activity of different spices and herbs.

Himalaya International is setting up Functional Food project at Keshwana,near Behnor,Jaipur.Liv-52, Triphala and Amalika are important herbal products of Rajasthan (Bhatia and Jain; Verma *et al*., 2011).

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Short Communication

ROLE OF BIO-FERTILIZERS IN HORTICULTURAL CROPS

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Abstract: The term bio-fertilizer is made up of two words "Bio" means living and "Fertilizer" means a product that provides nutrients in usable form. But as a product Bio-fertilizer does not contain any significant quantity of nutrients itself. It contains mainly live bacterial or fungal cells, which on application helps in fixing or solubilizing the nutrients present in air or in soil. These are natural fertilizers.

Keywords : Bio-fertilizers, Horticultural crops

INTRODUCTION

Continuous and unbalanced use of chemical fertilizer is leading to decreases in nutrient up take efficiency of plants resulting in decrease in yield. Frequent use of chemical fertilizers at a high rate also causes problem like soil health, deterioration and ground water pollution.

Types of bio-fertilizers

Some common by used Bio-fertilizers in horticultural crops (Fig.1)

1) Azospirillum

It is a non-symbiotic micro aerophilic bacterium commonly found in association with roots of horticultural crops. Its useful characters include high nitrogen fixation capacity and tolerance to high soil temperature. They fix nitrogen in the range of 10 – 40 kg per hectare. *Azospirillum* inoculation helps the plants in better vegetative growth and also in saving inputs of nitrogenous fertilizers by 20 – 30%. It is also well suited for plants raised through nursery.

2) Azotobactar

It is a free living nitrogen fixing bacteria, fixing N equivalent to 25 – 30 kg N/ha. It also produces hormones like IAA, gibberellins, vitamins etc. Benefits are – enhanced branching of roots, up take of NO_3, production of plant growth hormones.

3) Rhizobium

The largest contribution of biological nitrogen fixation is derived from the symbiosis between legumes and species of *Rhizobium*. It is a symbiotic leguminous N2 fixer. There is now definite evidence to show that legumes particularly the common bean (*Phaseolus vulgaris*), the most important legume for human consumption, respond positively to the inoculation with rhizobia and contribute to the nutrient status of the soil.

4) Phosphate Solubilizers

The phosphate solubilizers containing bacteria or fungi may convert insoluble form of phosphate to soluble form by producing organic acids, in general about 15 – 25% of insoluble phosphate can be solubilized. These fertilizers play a significant role in solubilizing insoluble phosphate. Around 95 – 99% of the total soil phosphorus are insoluble which are not directly available to the plants. Several soil bacteria; particularly *Pseudomonas & Bacillus* and fungi *Pencillium & Aspergillus* possess the ability to bring insoluble phosphate in soil into. Soluble forms by secreting organic acids such as acetic formic prop ionic, lactic, glycolic, fumaric and succinic acids. Their inoculum is available in packets of 200 gr similar to that of *Rhizobium*. They can be mixed with FYM and applied to soil.

5) Mycorrhizal fungi (VAM) Vesicular – Arbuscular Mycorrhizae

Mycorrhiza literally means "Fungus root" coined about a century ago. It is an association of plant roots with certain fungi. It is a symbiotic, which means that both plant and fungus benefit from the relationship. The fungi grows within the cortex of roots and send thread like hyphae out into the soil. This fungi extracts nutrients from the soil. Mycorrhiza is more beneficial when new land is brought under cultivation. Mycorrhiza greatly increases the uptake of nutrients especially P & N from deficient soils. It may also mobilize micro-nutrients such as Copper, Zinc, Iron and also helpful in biological control of root pathogens.

E.g. Papaya, Mango, Citrus, Banana, Grape, Pineapple and all vegetables are benefited by VAM inoculation. The most common method of application in sprinkle powdered inoculant on the roots at transplanting time or to add inoculant to soils just before seedling in transplanted crops. e.g. Onion, Tomato, Chilli, Ornamentals inoculation should be done at sowing time in the mother beds.

Role of bio-fertilizers (table 3)

a) In Vegetable Production (Table 2)

Bio-fertilizers are not alternatives to inorganic fertilizers. But they are useful in increasing yield, quality and production. Bhattacharya & Jain (2000) reported increased yield of many crops by use of P – Solubilizing Bio-fertilizers.

Application of Bio-fertilizers viz. *Azospirillum* and Phosphobacteria are known to increase the yield of various vegetable crops like Tomato, Brinjal, Onion, Chillies and Cabbage etc. Thilakavathy and Ramaswamy (1999) reported that application of *Azospirillum* and Phosphotika on the Onion seed bulbs and soil gave an increased yield of 18.3% as well as saved 25% inorganic fertilizers. In another experiment of bio-fertilizers on vegetable and seed yield of cabbage cv. Golden Acre should the best result on vegetable as well as seed yield by application on of 60 kg nitrogen in combination with 50 kg *Azotobacter* culture per hector (Verma et al. 1997).

The Regional Bio-fertilizer Development Centre, Nagpur conducted experiments at farmer's fields with respect *Azotobacter* and phosphate – solubilizer application on Okra, brinjal, chilli and cauliflower, the application of *Azotobacteres* increased the yield of Okra, brinjal, chilli and cauliflower to the tune of 8.3, 8.9, 15.7, 10.3, 6.2% respectively while the respective response of phosphate solubilizers on brinjal and cauliflower were 10.0 and 7.3%. Significant improvement the growth yield, nutrient uptake, dry matter and vitamin C contents in several vegetable crops were observed on bio-fertilizer application (Table-2).

b) In Fruit Production

Some innovations in this field are as follows :

Banana Suckers are placed directly in the pits filled with the VAM inoculums in FYM base. Application of phosphorus fertilizers was found to be reduced by 25 – 50% depending upon the crop.

Ashok Kumar and Shaumuguvelu (1980) conducted an experiment on the effect of *Azotobacter* as Nitrogen fixer on banana. They observed that inoculation of *Azotobacter* and Urea Spray increased the plant height girth and sucker production.

Jeeva *et al.* (1988) found that inoculation of *Azospirillum* in combination with nitrogenous fertilizers increased yield upto 13.1% in banana cv. Poovan and saving upto the extent of 65 kg/ha. of N.

Singh (1999) observed that total number of fruits in the mango genotype "MDCH – 1" and guava cv. L – 49 and 'Allahabad Safeda' were much higher in plants treated with bio-fertilizers.

Overall Benefits Due to Bio-fertilizer Application

1) It helps to increase availability of nutrients, especially Nitrogen and Phosphorus.
2) It can replace 20 – 50% of Chemical fertilizers viz. Nitrogenous and Phosphotic fertilizers.
3) Increasing farm productivity, generally 10 – 40% in grain yield and 15 – 30% in vegetative growth.
4) It helps by decomposition of plant residues and improving C/N ratio of soil, soil texture and structure and water holding capacity.
5) Bio-fertilizers are cheaper than the chemical fertilizers.
6) It helps in stimulating plant growth in general and roots in particular as they secrete various growth hormones provide better nutrient uptake and increased tolerance towards drought and moisture stress.
7) Some organisms also secrete some fungistic and antibiotic like substances that reduces the incidence of certain diseases and increase disease resistance efficiency.
8) Mobilize Micronutrients like Zinc and Copper.
9) Enhance Chlorophyll content favouring a higher photo-synthesis rate.

Economics of bio – fertilizers

1. Saving of 40 – 50 kg inorganic nitrogen per hectare,
2. 1 ton *Rhizobium* inoculant is equivalent to 100 tonnes of nitrogen.
3. 1 ton of *Azotobacter & Azospirillum* each is equivalent of 40 tonnes of N.
4. 1 ton of BGA is equivalent to 2 tonnes of N.
5. 1 ton of Phosphate solubilizers is equivalent to 24 tonnes of phosphorus pentoxide.

Locations of Several National and Regional Bio-fertilizers Production and Development Centres (After DGTD, 1989) (Table 1)

1) National Bio-fertilizers Development Centre, Ghaziabad, U.P.
2) Regional Bio-fertilizers Development Centre (North), Hissar, Haryana.
3) Regional Bio-fertilizers Development Centre (Central), JNKVV, Jabalpur, M.P.
4) Regional Bio-fertilizers Development Centre (West), MPKV, Pune, Maharashtra.
5) Regional Bio-fertilizers Development Centre (South), USDA, Banglore, Karnataka.
6) Regional Bio-fertilizers Development Centre (East), QUAT, Bhubaneshwar, Orissa.
7) Regional Bio-fertilizers Development Centre (North East), ICAR, Complex for North-eastern Hill Region, Shillong, Meghalaya

Fig. 1. Types of bio-fertilizers

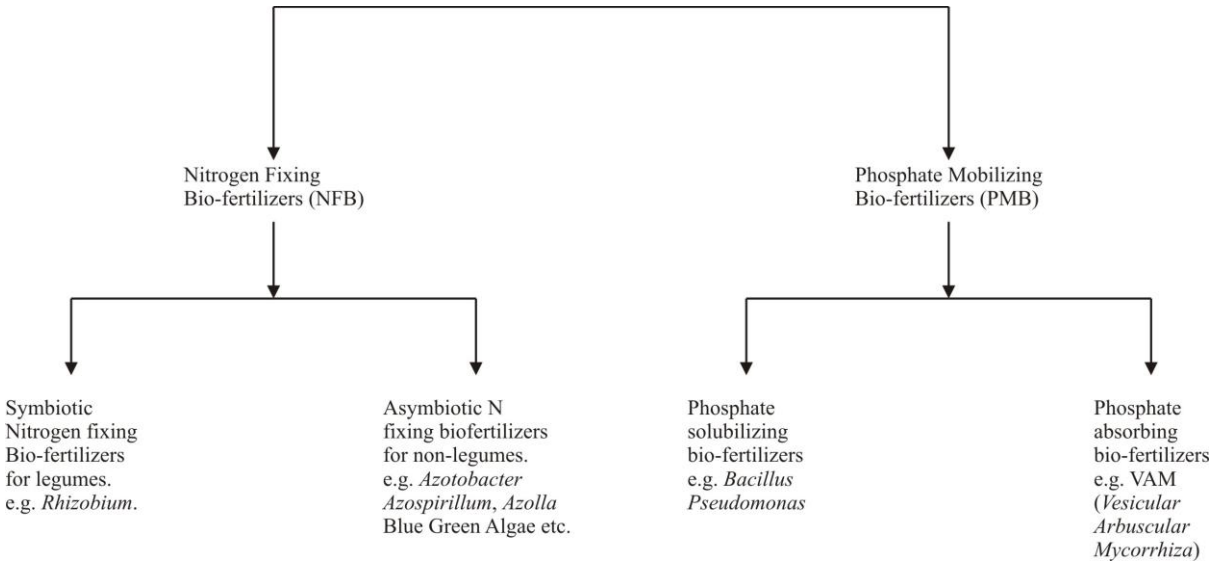

Table 1. Number of Bio-fertilizer Production Units Located in different States/Union Territory.

Sl. No.	Name of the State/ Union Territory	Total No. of BFPU	Total BFP capacity Tons/Yr./State	Distributed Pattern of BFPU.
01	Andhra Pradesh	4	165	SG(1) + AU(1) + P(1) + F1(1).
02	Assam	2	90	Al(1) + AU(1)
03	Bihar	2	20	AU (2)
04	Delhi	1	75	IARI
05	Gujarat	3	300	Fl(2) + MFD(1)
06	Haryana	2	125	Gl(1) + AU(1)
07	Himachal Pradesh	1	10	SG (1)
08	Karnataka	6	150	AU(2) + SG(1) +
				Gl(1) + P(2).
09	Madhya Pradesh	4	450	Gl(1) + Al(1) +
				MFD(1) + SG(1)
10	Maharashtra	5	250	Gl(1) + Al(2) +
				AU(1) + P(1).
11	Manipur	1	50	Gl (1)
12	Orissa	2	125	Gl(1) + Al(1)
13	Punjab	1	75	Gl(1) + Al(1)
14	Rajasthan	2	100	Al(1) + AU(1)
15	Tamil Naidu	9	450	SG(4) + AU(1) +
				Fl(3) + P(1).
16	Uttar Pradesh	13	300	Gl(1)+SG(10)+AU(2)
17	West Bengal	4	150	AU(1) + P(3)
	T o t a l	62	2885	

Abbreviations : AU = Agriculture University, A1 = Agriculture Industries, BFPU = Bio-fertilizer Production Unit, BFP = Bio-fertilizer Production, F1 = Fertilizer Industries, G1 = Government of India, MFD = Marketing Federation, P = Private, SG = State Government.
Source : Singh (1993)

Table 2. Doses of Bio-fertilizers for Vegetables.

Vegetable	**Bio-fertilizers for N & P**	**Method of application**	**Quantity of N Bio-fert. Required/ha.**
Potato	*Azotobacter* + P.S.M.	Soil/seed treatment.	4.0 – 5.0 kg.
Raddish Spinatch, Okra.	"	"	0.4 – 0.06 kg.
Turnip, Carrot	"	"	0.02 kg.
Onion, brinjal, Cauliflower, cabbage, Tomato, Chilli.	"	Seedling treatment.	1.5 – 2.0 kg.

PSM – Phosphate – Solubilizing Bacteria.

Yield increase in Vegetables at Farmers fields at different locations.

The Regional Bio-fertilizer Development Centre, Nagpur, conducted Experiments of farmers fields during 1997 – 98.

Place	**Treatment**	**Crops**	**Yield**		**Increase in yiel over un-**
			Control (q/ha)	Treated (q/ha) treated (%)	
Umri Nagpur	*Azotobacter*	Okra	24.8	26.0	8.3
Ambada, Narkhed	P.S.M.	Brinjal	125.0	137.5	10.0
Tivara, *Ajotobacter*	"	"	190.0	220.0	15.8
Amarvati, Nagpur	"	Chilli	14.5	16.0	10.3
Sweagram, Bopapur,	P.S.M.	Cauliflower	34.0	36.5	7.35
Chikhali, Katol Parshivani	"	Okra	23.4	25.5	8.97

Source : RBDC, VCA Complex Nagpur (1997 – 98)

Table 3. Bio-fertilizers for Horticultural Crops.

Vegetable/Fruit	**Microorganisms**	**Method of Application**
Legumes :		
Beans, Green	*Rhizobium legumino-*	Seed treatment,
pea, Dolichos	*sarum*, bv. *Phaseoli*	broad casting.
and Cowpea.	*Rhizobium* spp.	
Non – legumes :		
Tomato, Brinjal,	*Azospirillum, Azoto-*	Seed treatment, nur-
Chilli, Capsicum,	*bacter, Bacillus spp*,	Sery application and
Okra.	Phosphate solubili-	Seedling dipping.
	zing bacteria.	
Fruit Crops :		
Mango, Citrus,	VAM Fungi, *Glomus*	Nursery application
Papaya, Banana,	*mosseae, Azospirillum*	and to be applied
Grape and	and Phosphate – Solu-	in pits while
Pomegranate.	bilizing bacteria.	transforming

Nutrients Fixed/Made Available by DIF Microorganisms.

Microorganism	**Associated with**	**Dose (kg/ha/year)**
Rhizobium-N-Fixing	Leguminous Vegetables	30 – 180
Azospirillum and	Non – leguminous	30 – 80
Azotobacter-N-Fixing	Vegetables.	
Phosphate-Solubilizing	Transplantable Vegeta-	Solubilizes 40–100
Microorganisms.	bles and Fruits.	Of Fixed P.
Mycorrhiza.	All Vegetable and	
	Fruit Crops.	

CONCLUSION

The population pressure, water loss, soil erosion, floods, saline and alkaline soils, weed and pest damage are considered to be the main indicators of unsustainability. Hence, supply and judicious use of production factors play a decisive role in the sustainable growth of agricultural production. We have discussed on Bio fertilizers and can conclude that it is an economic, ecofriendly system, which attempts to provide a balanced environment, maintains soil fertility, control diseases and produce safer and qualitative food stuff. However technologies like organic farming or integrated management systems need to be assessed to their location, specific applicability and adaptability to bring about better sustainability. Over all organically grown food may not put more nutrients into once body but will surely optimize the health and production of inter-dependent communities of soil life, plants, animals and people. When one buys certified organic food and products, the money you spent cast a vote for a healthier planet.

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PROBLEMS EXPERIENCED BY RURAL WOMEN ENTREPRENEURS

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Abstract: The study was conducted in purposively selected block of Indore district of Madhya Pradesh during 2009-10 in order to know the entrepreneurial behaviour of rural women in terms of their socio-economic, psychological and family background attributes. The results of the study revealed that dual responsibility, lack of resources, poor family support, and late payment by clients, mobility and marketing constraints were the major constraints perceived by majority of the rural woman entrepreneurs.

Keywords : Indore, Rural women, Rural population

INTRODUCTION

Global changes have created economic opportunities and women entrepreneurs have emerged as a distinct class. Entrepreneurship is the dynamic process of creating incremental wealth. The wealth is created by individuals who take the major risks in terms of equity, time and career commitment of providing value to some product or service (Kuratko and Richard, 2001).

The women folk can easily be considered as backbone of any nation and better half of the men in almost all spheres of community development, of which India is not an exception. Rural women, who constitute about 50 per cent of total rural population, play an active role in all spheres of economic life and contribute richly towards national income. The concept of developing rural women entrepreneurship lays emphasis on the utilization of women labour force productively in generating income for their livelihoods, alleviating rural poverty, and in reducing negative social effects of unemployment and under employment. If women obtain better access to resources, education, and technology, they can and will create their own jobs or make their jobs more productive and remunerative. This will ensure a better participation of women folk in the process economic of growth of the nation. Therefore, measures to develop entrepreneurship among women are critical, because such efforts, even at a low rate, will definitely improve their status in the society.

In Indore district of Madhya Pradesh, the rural women are actively involved in various enterprises through formation of Self- Help- Groups. Hence, the study was conducted to explore the problem faced by the rural women in Indore block of Indore district of Madhya Pradesh.

MATERIAL AND METHOD

The study was conducted in Indore block of Indore district of Madhya Pradesh which was purposively selected, because it served a great deal of convenience for the research worker in terms of accessibility, ease of rapport building, time, money, and efforts. A list of the villages was made around a town or kasba, where market facilities and inputs are available for the enterprises. Care was taken to select only those villages which were well connected by road to nearby towns. Six villages were selected purposively from the selected block. A list of rural woman entrepreneurs was made from each village. A total of 60 respondents (ten respondents from each village) were selected for the study by using simple random sampling method. All the respondents were individually interviewed using pre-tested interview schedule.

RESULT AND DISCUSSION

Constraints experienced in the implementation of their enterprise as perceived and prioritized by rural woman entrepreneurs are presented in table 1. This study revealed that dual responsibility was ranked first among of rural woman entrepreneurs (96.66%). Lack of resources was ranked second. Poor family support was next in order of importance. Lack of awareness (78.33%) was ranked fourth followed by late payment by clients (75%), mobility constraints (70%), marketing constraints (65%), non payment by clients (62%), and non availability of funds from institutional sources (53.33). Finally, the non availability of guarantor (41.66%) was ranked last. Similar findings were reported by Singh (2008).

CONCLUSION

It is concluded that dual responsibility, lack of resources, poor family support, late payment by clients, mobility constraints and marketing constraints were the major constraints perceived by majority of the rural woman entrepreneurs.

Assistant Professor (adhoc), **Scientist & * Research scholar*

Table 1. Constraints experienced by rural woman entrepreneurs in the implementation of their enterprise n=60

S. No.	Constraints	Frequency	Per cent	Rank
1	Dual responsibility	58	96.66	1st
2	Lack of resources	55	91.66	2nd
3	Poor family support	50	83.00	3rd
4	Lack of awareness	47	78.33	4th
5	Late payment by clients	45	75.00	5th
6	Mobility constraints	42	70.00	6th
7	Marketing constraints	39	65.00	7th
8	Non payment by clients	37	62.00	8th
9	Non availability of funds from institutional sources	32	53.33	9th
10	Non availability of guarantor	25	41.66	10th

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POST HARVEST LIFE OF TUBEROSE AS INFLUENCE BY GA_3 AND VARIETIES

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Abstract: An experiment was conducted to evaluate the influence of GA_3 and varieties on post harvest life of tuberose. GA_3 was applied to plants at two concentrations (GA_3 100 and 200 ppm) along with control (distilled water). Varieties comprised of two single cultivars namely Sikkim Selection, Phule Rajani and two double cultivars namely Vaibhav and Calcutta Double. GA_3 200 ppm produced pronounced affect on post harvest characters of tuberose. All the varieties exhibited significant differences for all the attributes.

Keywords: Tuberose, GA_3, Cultivar, Growth, Yield.

INTRODUCTION

Tuberose is cultivated on large scale in France, Italy, South Africa, USA, India and many tropical and subtropical areas. It has great economic potential for cut flower trade and essential ail industry (Sadhu and Bose, 1973) due to their great demand. Bhaskar and Rao (1998) also reported that gibberellic acid, at concentrations of 50 or 100 ppm significantly improved the vase life of cut tuberose spikes. Gibberellic acid at 100 ppm was the most effective in improving the water uptake, maintaining a better water balance. GA_3 at 100 ppm was found most effective for increasing the fresh weight of flowers and percentage of opened florets per spike. Hence present study was conducted with aim to influence the post harvest life of tuberose varieties with the application of GA_3.

MATERIAL AND METHOD

The tuberose crop bearing both single and double flowers was raised at the Horticulture Experimental farm of the Banaras Hindu University, Varanasi during 2008 to 2009. Tuberose bulbs of uniform size were planted at a spacing of 25×25cm in $1m^2$ plot. GA_3 was applied at two concentrations 100 and 200 ppm along with control using distilled water. First spraying was performed at 35 days after planting and second spraying was applied at 50 days stage. Varieties comprised of two single cultivars Sikkim Selection and Phoole Rajani and two double cultivars Vaibhav and Calcutta Double. For recording of vase life parameters spikes were cut in the morning, above 20cm basal end. Basal 10cm stems were re-cut under water and the stems were put in distilled water for observations. Observations on various vase life parameters were taken carefully and analyzed statistically each character is presented in table 1.

RESULT AND DISCUSSION

Vase life of tuberose was highly affected by GA_3 treatments and varieties. Variations in vase life may be attributed to the differential accumulation of carbohydrates due to varied leaf production and sensitivity of cultivars to ethylene. In turn variations in these aspects might be due to genetical makeup of plants (Kamble *et al.*, 2004). GA_3 200 ppm significantly enhanced percentage of opened floret and water uptake however maximum vase life (days), days taken to 50% flowering. However, days taken to first floret withering was recorded maximum with GA_3 100 ppm treatment. The earlier work was carried out by Bhaskar and Rao (1998), Nagaraja *et al*. (1998) in tuberose are also in congruence with these findings. GA_3 at 100 ppm concentration produced maximum vase life (days). Three Asiatic hybrid lily cultivars Vermeer, Vivaldi and Marseille exhibited longevity of spikes in vase and reduction of leaf chlorosis with GA_3 treatment (Ranwala and Miller 2002). Cultivar Vaibhav was found most effective to vase life. Cv. Calcutta Double had taken maximum days to 50% flowering. Cv. Sikkim Selection exhibited maximum percentage of opened floret and Cv. Calcutta Double consumed highest quantity of water under vase life and maximum days to lowest first wither. GA_3 influenced the water uptake, vase life, fresh weight and floret opening in tuberose cv. Pearl Double (Kumar and Singh 2004).

Hence it can be concluded that all the post harvest parameters of tuberose were significantly affected by GA_3 treatments and varieties.

Table 1. Influence of GA_3 and varieties on vase life.

Treatment ppm	Vase life (days)	Days taken to 50% flowering	Percentage of opened floret	Total water uptake (ml)	Days taken to first floret in wither
$GA_3$100	10.80	6.17	51.71	88.99	4.24
$GA_3$200	9.30	7.56	54.39	90.79	3.17
Control	9.31	5.45	49.76	80.39	3.38
S.Em±	0.07	0.077	0.10	0.073	0.046
C Dat 5%	0.17	0.19	0.25	0.179	0.11
Cultivars					
S.Selection	7.77	5.61	56.37	27.99	2.41
Phule Rajani	9.07	6.28	46.90	83.19	2.87
Vaibhav	11.47	6.01	55.04	109.80	3.94
C.Double	10.9	7.66	49.51	125.31	5.17
S. Em ±	0.08	0.08	0.12	0.085	0.054
CD at 5%	0.19	0.21	0.29	0.206	0.13

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SCREENING OF OKRA GENOTYPES BASED ON LEAF SHAPE INDEX

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Abstract: Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable crop in the tropics and the subtropics. For characterization of diverse okra genotypes morphological characters play an important role.

Keywords: *Abelmoschus esculentus,* Genotypes, Okra

INTRODUCTION

Expression of leaf shape in okra is one of the important components and all varieties do not fall in one category. Bhutani *et al.* (1966) proposed a simplified index known as 'leaf lancination index' in cotton, which expresses the leaf shape as recognizable as conventional leaf shape indices on the basis of the above results. Arumugam and Muthukrishnan (1977) found out lacination indices by which okra cultivars can be classified broadly based on lacination index. The association of lacination index with Index 'C' (Sinus length / leaf length) and Index 'D' (lobe width / leaf length) are highly significant and negatively correlated while between Index 'C' and Index 'D', the co-relation co-efficient is positively significant. Hence, it is enough that the 'lobe length' and 'leaf length' alone can be measured for determining the leaf shapes of different cultivars of Okra.

In the present investigation fifteen genotypes of okra were grown under two seasons viz. Summer and rainy during 2005 at the Agricultural Research farm, Baruipur of Calcutta University. The experimental layout was randomized Block Design having three replications of each genotype. The plot size was kept at 2 mt x 1.5 mt for both the seasons. Spacing of 30 cm x 30 cm for summer crop and 50 cm x 40 cm for rainy season crop was taken ensuring 24 plants and 20 plants per plot in the respective seasons. Twenty leaves from each genotype were taken at random and measured. The leaves on the main stem were sampled from ninth and eleventh node when the plants came into flowering as the leaves from 7^{th} node onwords are representative of the shape and the size of the variety. The measurements included i) Lobe length (I) – length of the median lobe denoting the lobe exertion from the sinus, ii) Leaf length (L) – leaf length callus spot to leaf tip. The lancination index (LI) was the ratio of lobe length (I) / leaf length (L). Based on LI, four types of leaf shapes are recognized in okra.

a) Broad leaves with LI values from 0.50 to 0.60.
b) Intermediate types with LI values from 0.61 to 0.70.
c) Narrow leaves with LI values from 0.71 to 0.81.
d) Lacinated leaves with LI values of 0.80 and above.

The lacination index varied from 0.70 – 0.87 in the fifteen different cultivars (Table 1), out of the these four were having lacinated leaves with LI values of 0.81 and above while six cultivars were having narrow leaves with LI values ranging from 0.72 – 0.79 under both the environments. Four cultivars *viz.* 'Parbhanikranti', 'Sagun', 'Makhmali' and 'Mahyco–10' were seen to have lacinated leaves during summer and narrow leaves during rainy season. Similarly, 'Pankaj' was having narrow leaves under summer environment and intermediate type of leaves under rainy environment. The variation may be due to the environmental effect, which masks the small differences among different genotypes resulting in variation in the character, which is usually discontinuous in nature. Leaf shape index is an oligogenic trait and here the genetic variability in the form of oligogenic complexes resulting from the linkage between major genes and polygenes can lead to variation in the segregating generations. Further that leaf shape in okra is having close association with resistance to major pests and diseases and photosynthetic activity of the plant as detailed out by Premnath and Dutta (1970). Those cultivars having higher lancination index *viz.* 'Parbhani kranti', 'Bhendi hybrid No. – 18', 'Sagun', 'Makhmali', 'Shamali', and 'HR – 1' will be having higher photosynthetic activity and thus enhanced average fruit weight.

The leaf shape indices in okra should be given due importance while carrying out systemic breeding programme as this character is very much inter – linked to resistance towards major pests and diseases and should invariably be included in future biotic resistance programmes.

Table 1. Leaf Lacination Index for fifteen okra cultivars grown Under two environments.

Sl. No.	Varieties	Env.	Lacination Index	Leaf Shape Classification
1.	Parbhani Kranti	S	0.87	Lacinated leaves
		R	0.79	Narrow leaves
2.	Bhindi hyb. No. 18	S	0.86	Lacinated leaves
		R	0.81	Lacinated leaves
3.	Sagun	S	0.84	Lacinated leaves
		R	0.79	Narrow leaves
4.	Satdhari Green	S	0.73	Narrow leaves
		R	0.77	Narrow leaves
5.	Ankur - 40	S	0.79	Narrow leaves
		R	0.80	Narrow leaves
6.	Makhmali (F_1)	S	0.82	Lacinated leaves
		R	0.80	Narrow leaves
7.	Arka Anamika	S	0.75	Narrow leaves
		R	0.76	Narrow leaves
8.	Sel-11 (Pankaj)	S	0.74	Narrow leaves
		R	0.70	Intermediate leaves
9.	Mahyco - 10	S	0.81	Lacinated leaves
		R	0.80	Narrow leaves
10.	Shamali	S	0.85	Lacinated leaves
		R	0.84	Lacinated leaves
11.	Bhindi Sel – 5	S	0.82	Lacinated leaves
		R	0.82	Lacinated leaves
12.	Sresta	S	0.78	Narrow leaves
		R	0.75	Narrow leaves
13.	Bhindi No. 101	S	0.78	Narrow leaves
		R	0.72	Narrow leaves
14.	Harita	S	0.76	Narrow leaves
		R	0.73	Narrow leaves
15.	HR - 1	S	0.85	Lacinated leaves
		R	0.82	Lacinated leaves

Note : S – Summer environment
R – Rainy environment

1) Broad leaves with LI values from 0.50 to 0.60
2) Intermediate types with LI values from 0.61 to 0.70
3) Narrow leaves with LI values from 0.71 to 0.80
4) Lacinated leaves with LI values of 0.80 and above.

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