Effects of Sodium Dodecyl Sulfate on chromosomal behaviour of certain *Allium* species

By
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DECLARATION

I, Ms. Thejasenuo Julia Kirha bearing Ph. D. Registration No. 578/2014 dated May 20, 2014 hereby declare that the subject matter of my Ph.D. thesis entitled 'Effects of Sodium Dodecyl Sulfate on the chromosomal behaviour of certain *Allium* species' is the record of original work done by me, and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. This thesis has not been submitted by me for any Research Degree in any other University/Institute.

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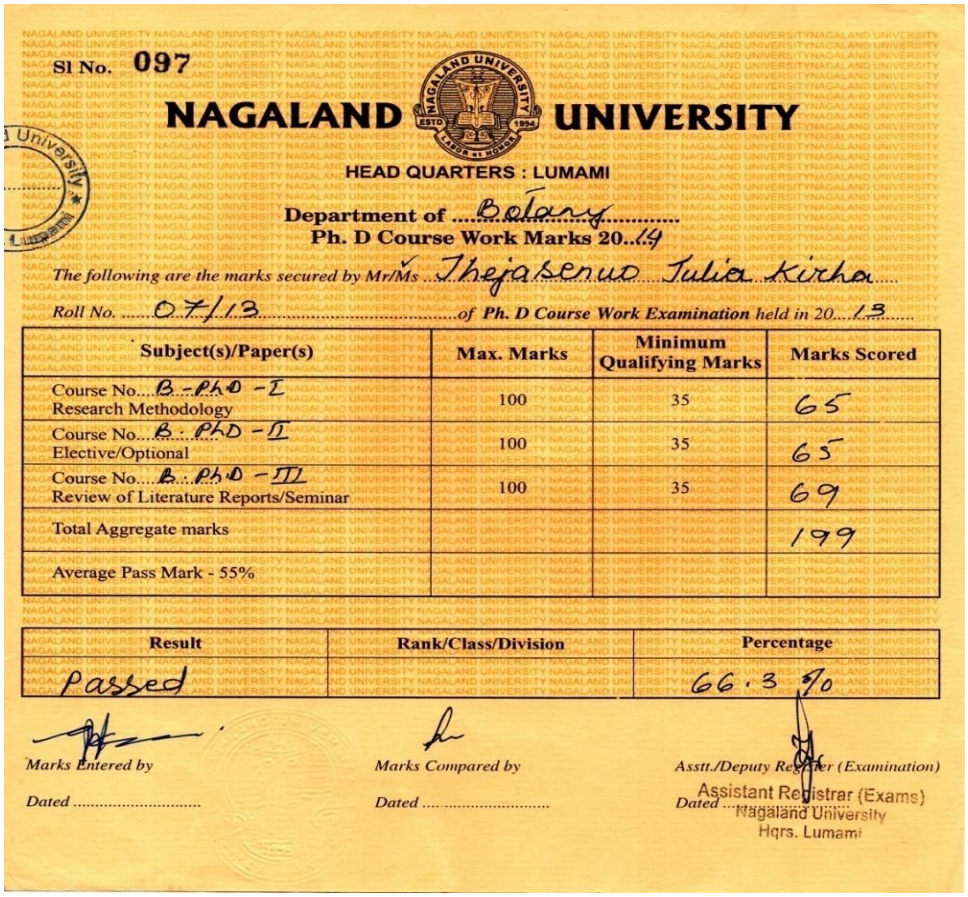
Ph. D. Course Work Mark Sheet and Certificate



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Subject(s)/Paper(s)	Max. Marks	Minimum Qualifying Marks	Marks Scored
Course No. B-PhD-I Research Methodology	100	35	65
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Result	Rank/Class/Division	Percentage
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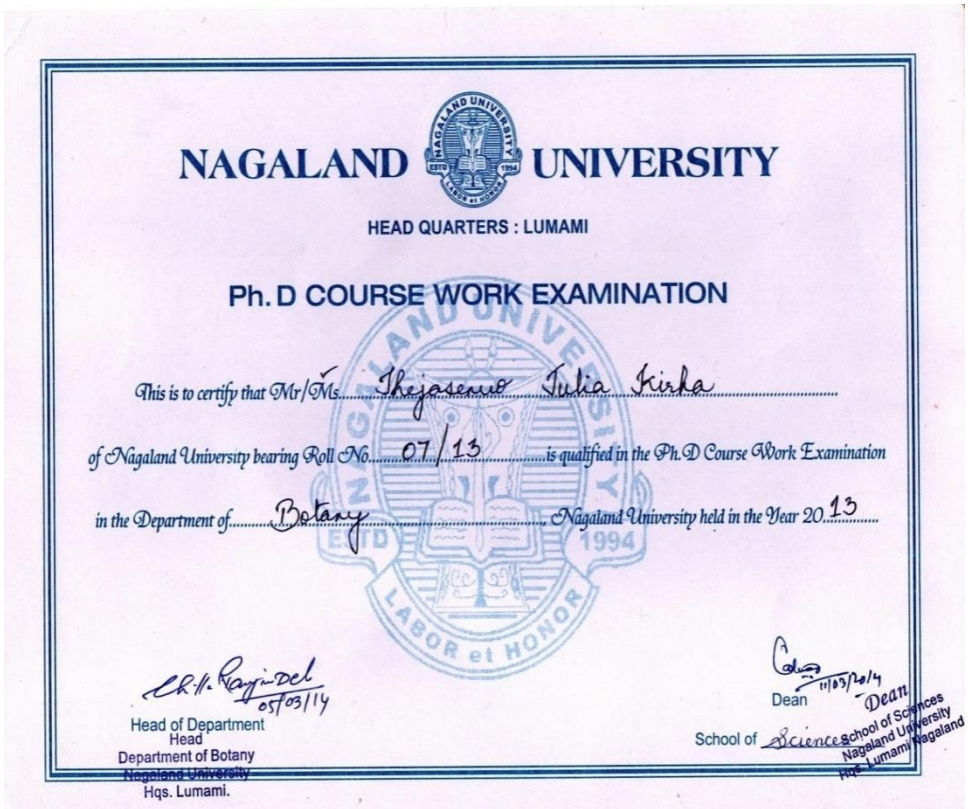

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Abbreviations

Abbreviation	Full Form
Abn%	Percent of abnormal cells
Abn_{Mean}	Mean value of abnormal cells
AE	Alcohol Ethoxylates
AES	Alcohol Ether Sulphites
AFM	Atomic force microscopy
AS	Alcohol sulphates
Biv	Bivalent
Bg	Bridges
Bk	Breaks
BKC	Benzalkonium chloride
BMI	Body mass index
BPAS	Branched primary alkyl sulfates
Cytm	Cytomixis
DC	Dividing cells
Dist.	Disjunction
DS	Dextran sulphate

DTAB	Dodecyltrimethy-l ammonium bromide
Frg	Fragment
FreqCA	Frequency of chromosomal aberrations
Gp	Gaps
HSV	Herpes simplex virus
Inter.chrm.	Intermingled chromosomes
LAS	Linear Alkylbenzene sulfonate
LDF	Laser Doppler flowmetry
Lg	Laggards
Loop form.	Loop formation
Loose end mate.	Loose end material
LPAS	Linear primary alkyl sulfates
NDC	Non-dividing cells
Nucm	Nucleomixis
Mac	Macronuclei
Mult	Multipolarity
Mnc	Micronuclei
MT	Metallothionein
MW	Molecular weights
PAF	Plate activating factor
PAGE	Polyacrylamide gel electrophoresis
PEGs	Polyethylene glycols
Rc	Ring chromosomes
SC	Stratum corneum
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

SDS Tol%	SDS salt tolerance
SEM	Scanning electron microscope
St	Sticky
Stry	Strayed chromosomes
TC	Total number of meiotic cells
TEWL	Transepidermal Water Loss
TOL	Toluene
Trans. chrm	Transition chromosome
Transl. chrm	Translocation chromosomes
TRIT	Tandem repeated irritation test
Twst.chrm	Twisted chromosomes
Uni	Univalent
Uneq.dis.chrm.	Unequal distribution of chromosomes
VFAs	Volatile fatty acids
WAS	Waste activated sludge

Chapter – 1

Introduction

From time immemorial, Cleanliness has been an important concern for human beings as a result production of soap and synthetic detergents came into being. Consequently, with time and rise of industrial revolution, uses of other detergents were realized. Currently, detergent industries are catering to the needs of industries and areas where detergents are now extensively used. Among different classes of detergents available, some of the detergents currently used in large quantities are Linear Alkylbenzene sulfonate (LAS), alcohol derivaties like Alcohol Ether Sulphites (AES), Alcohol sulphates (AS) and Alcohol Ethoxylates (AE) (Karsa,1992).

Sodium Dodecyl Sulfate (SDS) also known as sodium lauryl sulpfate (SLS), one of the most frequently used detergent is a primary alkyl sulfate which is a member of the Alcohol sulfate family. By means of oxo process, feedstock based Synthetic primary alkyl sulfates are derived from long-chain olefins yielding a mixture of linear and branched primary alcohols. Linear primary alkyl sulfates (LPAS) and branched primary alkyl sulfates (BPAS) produced by mixed alcohols sulfonation have exceptional detergent properties and are extensively used in heavy-duty detergent applications. SDS is denoted by molecular formula $NaC_{12}H_{25}SO_4$ and has a molecular weight of 288.38 g

mol^{-1}. SDS Synthesis is quite a simple process which involves the sulfation of 1-dodecanol followed by neutralization with a cation source. Its purification is obtained through repeated extraction and made commercially available in both broad-cut and purified forms (Dolkemeyer, 2000).

The synthetic surfactant development was encouraged due to synthetic soap shortage during World War I in Germany and its ineffectiveness in acidic as well as hard water (Kirk-Othmer, 1984). Since the late 1940's, synthetic surfactants especially anionic surfactant have been frequently used in higher proportions in industrial cleaning formulations as well as in consumer level (Haney, 1954; Ainsworth,1992). SDS synthesis process was first described in Germany (Lottermoser and Stoll, 1933). Linear primary sulfates are the simplest anionic surfactants, sulfated salt esters of paraffinic alcohols. Generally parent alcohols include 6-18 carbons, with lauryl sulfate as the most frequently used (Kirk-Othmer, 1984).

Lauryl sulfate in broad–cut distillation mixtures contains C_{10} to C_{18} where the sodium salt of C_{12} (SDS) is the most predominating component and is extensively used in household, industrial and manufacturing applications with its surface-active and micellar properties.SDS have the ability to lower surface tension of aqueous solutions and the ability to solubilize oils and fats or form micro-emulsions. These capabilities have been found to be applicable in a variety of ways such as an aid in manufacturing processes, biological research or an ingredient in consumer products. All alkyl sulphate particularly in C_{12} as an ingredient in consumer products, have cleaning, rinsing and foaming properties (Falbe, 1987).

SDS is extensively used in cosmetic and pharmaceutical industry as a detergent, wetting, emulsifying foaming agent and in drugs. It is also commonly used as anionic surfactants in shampoos. Due to its tendency to hydrolyse into lauryl alcohol and the

corresponding acid at low pH, “pH-balanced” shampoos are advice (Kirk-Othmer, 1984). It is also maintained in pharmaceutical chewing gums as solubilizer (Attwood and Florence, 1983).

Importance of SDS with PAGE is due to its capability to complex with proteins and charge differences are minimize and migrate them as anions (Shapiro *et al.,* 1967; Weber and Osborne, 1969). The insoluble proteins are solubilised and facilitated by SDS (Shapiro *et al.,* 1966) and also helpful in eletrophoretic studies (Maizel, 1966). In the second dimension during two-dimensional electrophoresis, SDS solubilized more plant polypeptides as compared to non-ionic surfactant (Booz and Travis, 1981). Certain limitations have been observed in spite of the usefulness of SDS-PAGE. According to (Leach *et al*., 1980) it favoured linear polypeptides, while, Huang and Mathews, 1990 reported polypeptides larger than 5000 Da were only useful.

Other uses of SDS included as a capability to form micro-emulsions by lowering surface tension at liquid-solid interfaces has been widely used in electroplating especially nickel and zinc (Kirk Othmer, 1984). SDS is used as an adjuvant in insecticides, an emulsifier and a wetting agent (Piper and Maxwell, 1971), also as an emulsifier and penetrant in varnish removers (Kirk Othmer, 1984). It is also used to increase the flow-ability of injection moulded explosives formulation and as an antifoaming agent in solid rocket propellants. Since, SDS has ability to disrupt lipid bilayers of cell (Kalmanzon *et al.,* 1992), it has been investigated to be used as shark repellent at 100ppm (Smith, 1991), because disorientation and withdrawal symptoms results in shark’s when its chemical and sensory systems are affected.

SDS or SLS is usually used in soaps, detergents, cosmetics, shampoos, as a leather softening agent, as flocculating and de-inking agent, engine degreasers and in paper industry, importantly used in nasal and ocular drug delivery, in trans-epidermal, to

boost intestinal absorption of drugs in animal models and in biochemical research in the laboratories. Now days, it is commonly used in biochemical research involving electrophoresis, protein extraction and isolation, SDS–PAGE, DNA extraction and lysis of cells (Hauthal, 1992).

SLS are generally used in SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) technique. The molecules loose their native shape or conformation due to the compounds disturbance or denaturation of the non-covalent bonds in the proteins. SLS anions also bind to the main peptide chain at a ratio of one SLS anions for every two amino acids residues. A negative charge is imparted on the protein that is proportional to the mass of that protein (1.4g SLS/g protein). The negative charge is found to be significantly greater as compared to the original charge of that protein. The protein unfold into a rod like shape due to the electrostatic repulsion created by binding of SLS hence, eliminates differences in shape as a factor for separation in the gel (Loffler and Effendy, 1999).

In haemoglobin analysis, Sodium lauryl sulfate is used. A confromational change is caused when the hydrophobic group of SLS acts upon the globin subunits, while SLS hydrophilic group binds with oxidised iron subunits and produces a stable reaction product which is analysed to give a haemoglobin value used as a part of a complete blood count. Brain tissue is cleared using SDS by the removal of lipids accountable for light scattering (Shen and Helen, 2013).

In aquatic and mammalian toxicological investigation, SDS is frequently used as a reference toxicant and a model surfactant. In aquatic toxicity testing, SDS is the first reference toxicants requisite by governmental regulatory agency and also in oil dispersants toxicity evaluation (Environment Canada, 1973; Environment Canada, 1984; Lee, 1980). Recommendation of SDS as the reference toxicants due to its fast–acting,

consistent and non-selective in its toxicity (La Roche *et al.,* 1970), while in oyster larvae stocks, due to variability in test results made it unsatisfactory for quality assurance testing (Cardwell *et al.,* 1977).

Biocide, a potential effective topical microbicide is represented by SDS, which can also possibly prevent or inhibit various enveloped and non-enveloped viruses such as HIV, herpes simples viruses and the semliki forest virus (Piret *et al.,* 2002; Piret *et al.,* 2000). The perception of sweetness is temporarily diminished by SLS (Adams, 1985) a common effect observed in toothpaste containing this ingredient.

A review of literature has been carried out to determine the effects and sensitiveness of SDS in animal models, humans and plant systems, if available as the following:

When treated with sodium dodecyl sulphate (SDS), Pyocin sheaths assembled into long rod structures in saline phosphate buffer however not in tris-HCL buffer. More NaCL or 0.05 M was required in the mixture for the formation of long rods with optimal SDS concentrations 0.05 to 0.2%. Study of the morphology showed the formation of each long rod by aggregation of several single sheaths (Amako and Yasunaka, 1974).

In young adults, SDS has been shown to irritate the skin when its application are prolong and constantly exposed (more than an hour) (Marrakchi and Maibach, 2006). People with chronic skin hypersensitivity may be made worse by the application of SDS (Agner, 1991; Nassif *et al.*, 1994; Loffler and Effendy, 1999). Study on the application of SDS in animals has shown to cause skin and eye irritation. Therefore, for SDS preparation in laboratory purposes, a dust free “pellet” form of SDS is made available. Apththous ulcers commonly referred to as canker sores showed recurrence by SDS in toothpaste as suggested by several studies. However, these studies have been inconsistent. A preliminary crossover study of SLS in toothpaste, in 1994 showed a high significant number of aphthous ulcers as compared to SLS-free toothpaste (Herlofson and

Barkvoll, 1994). Further double-blind crossover study in 1996 (Herlofson and Barkvoll, 1996) and (Chahine *et al*., 1997) supported these results. However, no statistical significant difference in number of ulcers was observed in a double blind crossover a study published in 1999, 2012 (Healy *et al*, 1999; Shim *et al.,* 2012) but found a significant difference in ulcer duration and pain sores (Shim *et al.,,* 2012) faster healing of ulcers was experienced on Patients who used SLS-free toothpaste and less ulcers-related pain on average than patients who used SLS-containing toothpaste (Shim, 2012). Formally Johnson & Johnson sold Rembrandt gentle white toothpaste; SLS-free toothpaste (Deardorff, 2014; Graedon and Graedon, 2014). Also interaction with fluoride, the effect of fluoride to prevent dental caries may reduce due to SLS as suggested by some studies. This may be owing to SLS interaction with fluoride deposition on tooth enamel (Barkvoll, 1989).

SDS is known to cause harmful effects on humans, animals and microorganisms. These harmful effects are dependent on the intensity of detergent concentrations and length of exposure in the specimen. Some of the effects of SDS have been illustrated on the cell activity of humans, animals, fishes, microorganisms (both fungi and bacteria) as well as on plant cells. When an individual ingest about ≤150 gram of SDS produces serious damage to his health including contact dermatitis, redness, swelling, blistering, Respiratory irritation, difficult breathing and lung damage (Eadsforth *et al.,* 2006; Basketter *et al.,* 2004) SDS by the dermal route (LD50 = ~600 mg/kg bw) and (>1200 mg/kg bw) is detrimental in rabbit and guinea pig (Dyer *et al.,* 2000). Guinea pig skin sensitization predictive tests showed hyperplasia and local irritation at 10% of SLS concentration (Lindberg *et al.,* 1992; Wong *et al.,* 2004). Oral repeat exposure of SDS (100 mg/kg bw/day dose level) in Male Wistar rats increased in the level of cholesterol esters and phospholipids and reduced the levels of triglycerides and cholesterol esters

with local irritation of the gastro-intestinal tract (Miura *et al.,* 1989; Dunphy *et al.,* 2001). Different SDS concentrations (3, 5, 7, 8.5,10 and 15 mg/1) showed morphological changes in spleen, kidney and inhibitory effect on fertilization success of gilthead (*Sparus aurata L.*) while, it showed 50% mortality in juvenile turbots (*Scophthalmus maximus L.*) at 384, 190, 12 and 4 hrs respectively (Ribelles *et al.,* 1995; Rosety *et al.,* 2001). SDS affects the metabolism, swimming capacity and survival of *Cyprinus carpio* and *Centropomus parallelus* (Barbieri *et al.,* 1998; Rocha *et al.,* 2007).

SLS of low molarity 2 was exposed daily for 4days on the skin of 37 volunteers and measured and visually scored by on the first day (before exposed, TEWL 1) and 5 day of exposure (TEWL 5). (When water passes from the dermis through the epidermis and evaporates from the skin surface, this is known as Transepidermal Water Loss or TEWL-International Journal of Pharmaceutics). It was found that skin type, age sex, history of sensitivity to soap micosal atopy were negligible. Hence, individual susceptibility to weak irritants may be indicated by TEWL (Tupker *et al.,* 1989).
Sodium lauryl sulphate, before and after exposure were examined on 28 atopic dermatitis patients where, high basal transepidermal water loss observed compared to control (P less than 0.0001) and leaning towards increased basal skin thickness (p= 0.056) (Agner ,1991).

Skin susceptibility of patients with seborrhoeic dermatitis to surfactant irritation studied and compared between normal group and people with atopic eczema. Patients with seborrhoeic dermatitis and atopic eczema observed increased susceptibility to SLS-induced irritation (Cowly and Farr, 1992).

An investigation on the effect of temperature on the damaged effects of water and detergents on the skin was conducted. For 2 days twice daily about 10 min, participants had to immerse both their forearm into a detergent soap solution which were randomized

to immersion into 20ºC and 40ºC respectively. Other 10 participants immersed both forearm in sterile water, the result showed increased TEWL immersions into 40ºC SLS as compared to immersion into 20º C SLS (Ohlenschlaeger J *et al.,* 1996).

At different concentration and exposure some SLS was applied with a degree of skin irritation produced visual assessment, chromametry, Laser Doppler flowmetry and TEWL was measured for irritant reactivity to SLS atopics and non-atopics. In conclusion, predictive human test of atopics and non-atopics will give similar results for acute skin irritation (Basketter *et al.,* 1998).

A study to determine the densities of keratin 16(K16) and 17(K17) during the course of acute patch test (A patch test is a method used to determine whether a specific substance causes allergic inflammation of a patient's skin) reaction to SLS expressed by keratinocytes and to relate to the proliferative state of the epidermis assessed by Ki 67 immunolabelling (Ki-67 is a protein that in humans is encoded by the MKI67 gene or Ki-67 is a nuclear protein that is associated with and may be essestial for cellular proliferation. Moreover, it is related with ribosomal RNA transcription). A significant increase in number of dividing kiratinocytes 48 hrs and 96 hr reactions was observed concurrent with high level of expression of K16 and more moderate expression of K17. The factor which influence and control their expression differs (Willis *et al.,* 1998).

The evaluation of SLS and DS (dextran sulphate) in different marine models against herpes simplex virus (HSV) and HIV showed potent inhibitor of the infectivities of a variety of HSV-1 and HSV-2 strains. Hence, to check sexual transmission of HIV, HSV and other pathogens SLS could possibly represent as a microbicide (Piret *et al.,* 2000).

Repeated application of SLS (5%) and toluene (TOL) twice daily for 30 mins to the forearm of 20 volunteers produced skin irritant reaction indicated by skin redness and

increased TEWL. SLS/TOL and TOL/SLS application induced strong reactions. The study conducted showed mixed application of an anionic detergent and organic solvent has an additive effect on skin irritation (Wigger-Albertini *et al.,* 2000).

Benzalkonium chloride (BKC) along with sodium dodecyl sulfate (SDS) an anionic surfactant was conducted for Patch testing. SDS concentration of 20% was applied for 2hrs followed by 1% BKC to the same area. The result showed BKC on skin exposed to SDS attenuates the resulting irritant reaction (McFadden *et al.,* 2000).

To investigate the influence of a thermal stimulus on skin pre-treated with SLS 0.25% and 0.5% for 48 hrs seventy seven volunteers were patch tested. Degree of irritation and increased skin blood flow was observed to be significantly correlated after thermal stimulus depended on SLS concentration (Loffler *et al.,* 2001).

A study to determine a possible relation of skin response to SLS between forearm and back at a concentration of 0.125%, 0.25%, 0.5% and 1.0% were assessed by visual scoring and TEWL measurement. Both TEWL and visual scoring were observed to be well correlated with SLS concentration. Compared to the back a prominent reaction of the forearm was observed. By TEWL measurement, a patch test on the forearm for 48 hr with 0.5% SLS or visual scoring for 24 hrs after patch removal was recommended. For practical reasons 0.5% SLS may be adequate if SLS patch is placed on the back along with allergic patch test (Loffler *et al.,* 2001).

To determine the interrelationship between SLS concentrations (0.125%, 0.25%, 0.5%, 1.0% and 2.0%) with variation in exposure time (3, 6, 12, 24 and 48 hrs), patch testing was performed. More consistant and constant skin reaction, higher correlation between SLS concentration and skin reactions were observed (Aramaki *et al.,* 2001).

Evaluation using bioengineering method, a correlation between body mass index (BMI) and epidermal functions were conducted. Before and after irritant patch test with

sodium lauryl sulphate (SLS) and increased transepidermal water loss (TEWL), skin blood flow compared to control group. It was observed that skin sensitivity to SLS was not found to be correlated with BMI (Loffler *et al.,* 2002).

Anti inflammatory effects of leopoldine sulphate water with sodium lauryl sulpahet (SLS) on human skin was evaluated (Hercogova *et al.,* 2002).

Pruritogenic substances (histamine substance P, neurokinin A, neurokinin B, trypsin, plate activating factor (PAF) and serotonin) and saline as control were injected on the inflamed test sites and non treated sites on the opposite forearm. In both normal and inflamed skin of volunteers Histamined and substance P induced itch to the same degree pre-treated with SLS (1%) despite a stronger weak response in inflamed skin compared with a saline reference. Neurokinin A, trypsin PAF and serotonin only elicited itch in normal skin and neurokinin B. Neither elicited itch in normal or inflamed skin (Thomas *et al.,* 2002).

Possible beneficial effects of rice starch in water on impaired barrier function measured by TEWL. Volunteer whose forearm skin was irritated by SLS was exposed to rice starch which led to 20% improvement of the damaged skin. Atopic dermatitis patient's skin barrier function also improved. Therefore, rice starch as a skin repair bathing additive in powder or formulated in bath producers can be recommended (De Paepe *et al.,* 2002).

Reports on useful as well as adverse effects of anionic surfactants on the atmosphere area unit are critically mentioned. The function of anionic surfactant in the environment has been found to be ambiguous i.e., it can cause harmful result on living organisms with serious environmental pollution or help in the decomposition or removal of inorganic and organic pollutants. More studies need to be conducted to understand their physicochemical parameters, chemical structures, environmental impact and

biological activity. A substantial variety of knowledge area unit required for the event of recent anionic surfactants and for the productive application of the present ones to cut back the antagonist and to market helpful effects (Cserhati *et al.,* 2002).

Application of Epicutaneous patches along with SLS concentrations 0.25%, 0.5%, water and an empty test chamber for different time intervals (i.e., 12, 24, 48hrs) on the volar forearm. TEWL values remained increased for 24hr. TEWL values on water patch increase to 180 min and on empty patch sites for only upto 120 mins. However, TEWL decreased for all patches from 0 to 120 mins which suggest occlusive effect of TEWL in patch testing ends 3hrs after removal of test chambers (Friebe *et al.,* 2003).

A study to analyse whether patch testing with hydroxycitronellal combined with SLS caused stronger patch test elicitation reaction than with hydroxycitronellal alone. Forearm patch testing with 6 concentration of SLS plus hydroxycitronellal showed more positive patch test (+, ++ or +++) than forearm patch tested with hydroxycitronellal alone. Further, positive patch test on hydroxycitronellal forearm was observed on no day of patch test readings (p=0.0253) (Heydon *et al.,* 2003).

Study on the climatic condition and cutaneous reactivity to a detergent was investigated where epicutaneous patch testing with 0.5% SLS between January 2000 and December 2001 was applied on the forearm of 487 volunteers and assessed by TEWL measurement of a significant correlation between steam, pressure, temperature, absolute and relative humidity and increase TEWL recorded and most pronounced during winter and spring. The data confirms increase skin irritation during winter with cold and dry air (Loffler *et al.,* 2003).

Addition of 1N HCL and 1N KOH was observed to cause pH changes as a function of concentration for sodium dodecyl sulfate (SDS) and dodecyltrimethy-

lammonium bromide (DTAB). The pH values increased and decreased for SDS/HCL system and DTAB/KOH system respectively (Yüksel , 2003).

Under occlusive condition, twenty volunteers were applied with 1% SLS solution for 24hrs on the side of one forearm. Result showed stratum corneum hydration reduced immediately and hyper–hydration observed (Gloor *et al.*, 2004).

Skin reaction after repeated SLS test and two brief tests giving special attention to the recovery rate was studied. The measurement of TEWL and erythema of 29 volunteers after application of 0.03, 0. 1 and 0.3 % SLS for 6hrs, 3 days per week for over 3 weeks indicated a weak correlation between repeated test and brief SLS test when an increased TEWL or recovery rate was used respectively (r=0.04 and 0.26 respectively) or recovery rate (r=-0.01 and 0.42) (Koopman *et al.,*2004).

Toxicity of a very common and known compound in pharmaceutical products and detergent (surface active) from cosmetics markets was studied with different concentrations of detergents 0.5%, 1% and 3% the effects of an ointment base with SLS on a normal model with Sprange Dawley rats (Dehelean *et al.,*2004).

A study conducted on long term exposure to SLS induced irritation of the skin contact dermatitis at a concentration of 0.025% to 0.075% to demonstrate possible accommodation by visual scoring (Erythema and dryness) and bioengineering method such as TEWL chromametry, capacitance, and Laser Doppler flowmetry (LDF) and no evidence of sustained irritation or adaptive hyposensitivity after long term exposure (Branco *et al.,* 2005).

Hardening phenomenon is the adaptation of the skin to repeated influence of exogenous irritants. Before and after induction of a hardening phenomenon in stratum corneum (SC) was investigated, where 23 non atopic volunteers was induced with irritant contact dermatitis by 0.5% SLS repeated occlusive application over 3 weeks.

Comparison of SLS pre treated skin and normal skin was performed after 3, 6 and 9 weeks. TEWL increased after 2 weeks and decreased on the 3rd week. Ceramide 1($P<0.001$) increased significantly which was found to play a vital role against repeated irritation by acting as a protective mechanism (Heinemann *et al.,* 2005).

Study on the additive impairment of permeability function by mechanical irritation combining with SLS 0.5% or prolonged water exposure by occlusion as gentle irritation was conducted where volar forearms of 20 volunteers were exposed to irritation and occlusion with water or 0.5% SLS for 4days at a combined tandem repeated irritation test (TRIT). The degree of barrier disruption was affected by the choice of irritant under occlusion, time occlusion and order of tandem application. SLS (0.5%) and physical irritants such as friction, abrasive, grains, occlusion gives a significant irritant risk and therefore, should be minimized particularly when acting together (Fluhr *et al.,* 2005).

To assess the effects of short term exposure to air flow at different temperature (24 º c and 43 º) combined with SLS (0.5%) using TEWL measurement on 20 volunteers found impairment of barrier function and significant increase in irritation compared with that the produced by SLS alone produced sequentially by air flow and SLS (Fluhr *et al.,*2005).

To assess skin damaged by SLS on percutaneous penetration of polyethylene glycols (PEGs) of different molecular weights (MW) which was determined using tape stripping of stratum corneum (SC). (Tape stripping, a simple and efficient method for the assessment of quality and efficacy of cosmetical and dermatological formulations. The cell layers of stratum corneum after topical applications and penetration of formulations are successively removed using adhesive film). The skin of volunteers were pre-treated with 5% w/w SLS for 4hrs, 24hrs and patched with PEG for 6hrs. TEWL, diffusion coefficient for PEGs increase after SLS treatment. However, increase was smaller for

MW and between SC and water the partition coefficient of PEGs was larger and increase with MW, which decrease gradually with increase MW of PEGs (Jakasa *et al.,* 2006).

Investigation between baseline stratum corneum (SC) cytokinin level and response of skin to a single and repeated test were conducted with 20 volunteers after 24 hrs exposure to 1% SLS, TEWL and erythema were measured and over 3 week period after exposure to 0.1% SLS, (Interleukin) IL-IRA, IL-8 increased 10 folds and fourfold respectively while interleukia (IL) 1 alpha decreased by 30% after repeated exposure (De Jongh *et al.,* 2006).

To assess the anti inflammatory activity of *Poria cocos* in SLS experimentally induced ICD. POCO 3 concentrations in base cream DAC (amphilic emollient, German pharmacopocia) were tested using repetitive SLS for 4 days. Significant anti inflammatory activity was statistically observed for POCO. Which can be explained by its influence on pro-inflammatory enzymes, phospholipase A2 (Fuchs *et al.,* 2006).

1% SLS exposed to the skin for 24hr on the skin water barrier function was analysed with transepidermal water loss (TEWL) and inflammation quantified by erythema,was found to be dependent on the SLS penetration rate determined by SC thickeness (De Jongh *et al.,* 2006).

Investigation of the effects of sodium dodecyl sulfate (SDS) on hydrolysis and acidification of waste activated sludge (WAS) caused solubility of carbohydrate and protein improved and increased with SDS The enzyme activities of protease and alkaline phosphatise also improved by SDS (0.02-0.3g/g) but α-glucosidase and acidic phosphatase activities decreased at SDS dosage above 0.05 and 0.2g/g, respectively. Under room temperature, volatile fatty acids (VFAs) production was also enhanced which increased with the amount of SDS. Further, this study showed the effects of SDS

on the improvement of VFAs production and inhibition of methane production in WAS fermentation than due to pH variations (Jiang *et al.,* 2007).

A study was conducted to evaluate the role of MT genes in sodium lauryl sulphate (SLS) induced skin irritation by close –patch testing of 2.5% 5% 7.5% and 10% in distilled water on right dorsal skin of MT (-/-) mice for 24 hrs. The results suggested MTI and MTII genes to play a significant proctective role in SLS irritation (Ma *et al.,* 2007).

Transdermal fluid were sample in 9 volunterrs after 4 hrs 10% SLS exposure and 3 week repeated exposure (0.1% SLS) differential and concerted expression of various inflammatory mediators and markers were found to be induces due to repeated irritation (De.Jongh *et al.,* 2007).

Non–invasive tools such as laser-doppler flowmetry, TEWL and coineomerty were used to investigate human skin respond to alcohol–based disinfectant and detergents. Propanol based hand disinfectant (sterillent) its propanol mixture (2-propanol 45% w/w and 1- propanol 30% w/w), SLS 0.5% and distilled water irritative effects were quantified. Results of the alcohol based test preparations showed irritation on the skin while SLS detergent produced strong barrier disruption, erythema and dryness. However, no irritation produced when SLS and disinfectant are combined together (Slotosch *et al.,* 2007).

To asses potential water retension capacity of control emulsion and oil in water (O/W) emulsion consisting of ceramide 1, ceramide 3 or both, 15 women (age 20-30) pre-treated with SLS were applied the emulsion twice daily for 28 days. After 4 weeks, maximum increase in skin humidity and maximum decrease in TEWL was obtained. Therefore, it can be concluded that skin barrier functions improve with ceramide–containing emulsions as compared with untreated skin (Huang and Chang, 2008).

Exposure to water (vehicle) and 1% SLS under occlusive patch test for 24 hrs was studies in healthy volunteers enzymes involved in corneodesmosomes degradation and mRNA expression of keratinocyte differentiation markers was examined during repair phase i.e., 6 hrs to 7 days post exposure using real time reverse transcription PCR suggested involucrin increases at 6hrs and then normalised. Tranglutaminase 1 expression increase 2 folds after 24 hrs of SLS exposure. SLS exposed areas showed > 50% above compared to control. Skin barrier defects by SLS induced altered mRNA expression of keratinocyte differentiation markers and enzymes degrading corneodemosomes (Törmä *et al.,* 2008).

Thirty six healthy volunteers were applied SLS (3%v/v) under occlusion on the back for 24 hr to test the anti-inflammatory effect of pimecrolimus cream after damage of the skin barriers due to SLS. The test area was subsequently treated with pimecrolimus cream 1% for 24 hrs, 1% hydrocortisone in hydrophilic ointmentand vehicle alone for 3 consecutive days. The result showed significant reduction in SLS induced erythema. The pimecrolimus penetrate into the skin reducing the irritation induced erythema this shows pimecrolimus effective in the skin disorders with impaired function of the epidermal barrier (Engel *et al.,* 2008).

To investigate the physical properties of how skin change after a long term application of the acidic or alkaline pH skin care products pH and glycolic acid. The 20 healthy volunteers ventrical forearm were applied with skin care products for 5 weeks. SLS (1%) irritation and erythema by UV were measured TEWL of SC increased significantly on the applied site (pH 8) skin barrier were empaired and disrupted severely by 1% SLS exposure due to sensitivity to external stress and impaired by alkaline pH of SC. Therefore it is significant that skin care products pH is important for skin barrier homeostasis (Kim *et al.,* 2009).

Sodium dodecyl sulfate shown to activate purified latent broad bean polyphenoloxidase, whose characterization was further carried out in the presence and absence of SDS. Increased activated enzyme was observed with SDS concentration up to a maximum of 1.75 mM in a sigmoidal manner. Eliminated low pH, increased thermo-liability of the enzyme as well as increased binding of dihydroxy-phenylalanine, size and apparent molecular mass of the enzyme under size exclusion chromatography on high performance liquid chromatography were slightly altered all observed in the presence of SDS. Concentration of SDS when increased also observed increased electrophoretic mobility and intrinsic fluorescence of tyrosine and tryptophan residues in a complex way. Alteration in enzymatic and physical characteristics due to the capability of SDS activation of the enzyme suggest limited conformational change because even a small amount of SDS binding may bring about the activation of latent enzyme (Moore and Flurkey, 1990).

By means of photosynthetic activity and chlorophyll content in bean leaves, assessment on the effects of detergent for domestic use was investigated. For 21 days, a solution of domestic washing powder 0.60 g r/l was watered on the plants. Photosynthetic activity showed no more than 45% of that in control plant. Chlorophyll concentration rose non-linearly in plant leaves with increase in plant treatment duration. On 21st day highest change was observed and amounted to 12% (Branislav *et al.,* 2010).

Using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) techniques, investigation of the effects of surfactant on the morphology of ZnO crystals was conducted. The shape and size of ZnO particles were significantly modified due to cationic-anionic surfactants mixture. A variety of structures such as sheets, spheres, flakes, rods and triangular –like particles ranging from micro to nano were obtained (Ramimoghadam *et al.,* 2012).

Disposal methods such as biooxidation and bioleaching for copper mine tailings to recover metals and to cut back environmental threat of virulent components and the effect of several residual flotations like Sodium dodecyle sulphate (SDS) in copper mine tailings remain unknown. the effects of SDS on copper mine tailings by *Acidithiobacillus ferrooxidans* on 5mg/L and 10mg/L was conducted where 10 mg/L SDS inhibits *A. ferrooxidans* growth, biooxidation loss and change in mineral surface hydrophilicity. After 30 days of biooxidation, observation of the surface of copper mine tailings under scanning electron microscope (SEM) showed aggregates propose a mixture of chemical and biological oxidation during booxidation. This study indicates the recovery of *A. ferrooxidans* with 5mg/L SDS, however, Cu dissolution rate failed to improve (Dou *et al.,* 2013).

Sodium lauryl sulfate (SLS) often labelled as 'environmentally friendly' in cleaners shows low toxicity in a single species toxicity test. However, sensitivities to SLS, differs in organisms at the community level, which suggest for measurement of effects of SLS. Microalgae (*Chlorella* sp.) and invertebrate grazers (Benthic snail, *Elimia* sp. and Pelagic microcrustacean *Daphnia magna*) Communities were exposed to 0, 0.5 or 1.5 mgL^{-1} SLS, where the quality of water it remain within acceptable range for the organisms to survive. *Chlorella* sp. remained unaffected by SLS but *Daphnia magna* decreased in number. Therefore, this study proposed SLS to be toxic to *Daphnia magna*, which is a significant food source for invertebrates and fishes and may affect organisms at higher trophic levels (Stephanie and Shipley, 2014).

A study conducted to assess the effect of the reactive additives sodium sulfite, sodium dodecyl sulfate (SDS), and urea on the oxygen barrier, water vapour barrier, and protein solubility of whey protein cast films (Schmid *et al.,* 2017).

A lot of attention has been attracted by surfactant films on solid surfaces due to their scientific interest and applications like micro or nano scale templates for micro fluidic devices or surface treatment agents. Sodium dodecyl sulfate (SDS) an anionic surfactant along with various charged inorganic salts was spread on a glass substrate and dried to form an SDS thin film. For the observation of micro-structure of the SDS thin film Atomic force microscopy (AFM) was used. Pure SDS film formed patterns of long, parallel, highly-ordered stripes demonstrated by experiments performed. Because of the interaction between the cationic ion and the anionic head groups of SDS existence of the inorganic salt disturbs the SDS film structure. Morphology of the SDS film changes due to the greater electrostatic interaction with anionic head groups by divalent ion than the monovalent ion. Height of the SDS bilayer calculated was in line with the theoretical worth and also the addition of the large-sized monovalent particle would cause lowering the height of the adsorbed structures (Shen and Lee 2017).

Literature survey reports indicates that most of the work includes model such as animal, human, fishes, rats etc., which have positive as well as negative impact and a few work has been done on plant system. From the above studies conducted, it can be ascertained that affects of SDS impacts us and the environment one way or the other due to its numerous applications in our lives whether in big or small amount. The time period of its application has considerable role to play in the toxicity of this compound.

Literature survey also indicated that SDS is an important ingredient of many household articles which was generally used by us every day. It has been tried to trace out the amount of concentration used in this article, but could not. However, the concentration of SDS ingredient in famous toothpaste Colgate was able to be traced, used by almost each and every one and it was recorded 0.24% which is equivalent to 2400ppm. SDS concentration 10% is used in the SDS-PAGE which is equivalent to

10^5ppm. The involvement of SDS as ingredient in various articles and its high concentration prompted us to examine the genotoxic effect of SDS using *Allium* test.

In *Allium* test, *A. cepa* has been widely used to test the different chemicals, pollutants and pesticides for the determination of their toxic and mutagenic threats. In the present study, different *Allium* species has been used other than *A.cepa* to determine the mutagenic or genotoxic potential of SDS.

Because of various response of stress by many chemicals, the present study has been conducted using Sodium dodecyle sulfate (SDS) due to its involvement in house hold articles as a common ingredient and an emerging threat to the environment. The tremendous use of the chemical in daily household activity, in animals and plant system prompted us to determine the physiological as well as clastogenic effects of this chemical in plant genetic system of *Alliums*. Further, higher plants may supply an important hereditary test method for screening and scrutinizing the genotoxic effects of SDS. Therefore, it is necessary to measure the detrimental effects of SDS on DNA, RNA, proteins, enzymes, physiology, cytology and metabolism of plants, animals and mammals. Moreover, the field of environmental mutagenesis still requires further hard work to estimate thousands of noxious waste that are released every day in our environment.

Different *Allium* species (*A.ascalonicum*, *A.hookeri*, *A.tuberosum*, *A.porrum, A.chinense*) at three different concentrations (500ppm, 1000ppm and 1500ppm) was used in the present study to observe the said effects for 3 hrs on the cytogenetics with the following objectives:-

1. To determine the LD_{50} of SDS in *Allium* species collected from different parts of Nagaland.

2. To determine the chromosomal aberrations in *Allium* species at different concentration and time of SDS.
3. To study the effects of SDS on meiotic index in *Allium* species at different concentration and time of SDS.
4. To correlate the chromosomal aberrations of *Allium* species for sensitivity and resistivity of *Allium* species to SDS.

Chapter - 2

Materials and Methods

The whole plant of *Allium* species (*A. ascalonicum*, *A. hookeri, A. tuberosum, A. porrum* and *A. chinense*) were collected from different locations of Nagaland and maintained in the Department for their vegetative and floral morphological studies.

The GPS reading for the plant material collection site of *Allium* species from various locations of Nagaland recorded and provided in Table below:

***Allium* spp.**	**Vernacular name**	**Places visited**	**N´**	**E´**	**Elevation (m)**
A.ascalonicum L.	Thamara(Ang), Shamoro(Mao)	1.Jakhamavillage 2.Jotsoma village	1.25º58905´ 2.25º39.550´	94º12.65´ 94º4.899	1.1585m 2.1589m
A.hookeri Thw.Enum.	Sela(Ang), Atsuna(Sema)	Lumami village	26º12.563´	94 º26.599´	1028m
A.tuberosum Rottle.Ex.Spreng	Selaketsü(Ang)	1.Lumami village 2.Sema Settsü	1.26º12.510´ 2.26º13.490´	94º26.509´ 96º28.592´	1.1030m 2.1035m
A.porrum L.	Chümerie kezhauo(Ang)	Wokha town	26º05.8981´	94º15.833´	1474m
A.chinense G.Don	Khuvie(Ang), Khova(Chak)	1.Lumami village	26º12.570´	94º28.618´	1022m

The young developing buds/anthers were pretreated with PDB (Para-dichlorobenzene) for 3hrs. After washing in distilled water for 20 min, the young developing buds/anthers for meiotic study were fixed in carnoys solution (ethyl alcohol and glacial acetic acid 3:1)

at room temperature for 24hrs, followed by 70% ethyl alcohol at 4^0C for further use. The chromosomal analysis was done according to the conventional squash method (Sharma and Sharma, 1980).

Preparation of concentrations and treatment:

The different concentrations of SDS were prepared where 0.05g, 0.1g, 0.15g were dissolved in 100ml of water for 500ppm, 1000ppm and 1500ppm and applied to the anthers/ buds for 3h each to *Allium* species and recorded the data. The treatment without the concentration is considered as control.

Preparation of slides:

The anthers/buds removed from the fixatives or preservatives and rinsed the anthers properly with distilled water three times at the interval of five minutes or till to ensure that material is free from the acid. The anthers/buds were treated with enzyme pectinase for 30 min and rinsed with water 2-3 times. The buds were treated with 1N HCL for 30min, rinsed and stained in acetocarmine (2% w/v) for about 15 min and squash in acetic acid (45% v/v). Slides were prepared and cytological analysis was carried out and photographs were taken under Leica microscope.

Data record and observation:

The prepared ready slides were observed under the microscope for treatment and control and recorded the data on number of meiotic cells, LD_{50}, meiotic images, types of meiotic images, types of abnormalities, physiological, clastogenic, meiotic index, sensitivity and resistivity of different *Allium* species against the SDS concentrations.

Observation on meiotic cells:

The good slides (clean and debris free) were prepared and data were recorded on the total number of meiotic cells (TC) including total dividing (DC) and non-dividing

meiotic cells (NDC) from the five different slides with digital Leica microscope. The mean values were recorded for each *Allium* species.

***Observation on meiotic stages**:*

The good slides (dust and debris free) were prepared and searched or observed for good meiotic stages (leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase I, anaphase I, telophase I, metaphase II, anaphase II, telophase II and cytokinesis) and captured them with the help of digital Leica microscope for all the *Allium* species and reported as mean value and photographed.

***Observation on meiotic index**:*

The total number of dividing meiotic cells and total meiotic cells were used to calculate the meiotic index and reported in percentage for all the *Allium* species.

Formula used:

The following formulas are used to calculate and analyze the data:

$$\text{Meiotic Index (MI)} = \frac{\text{Total number of dividing cells in control and treatment}}{\text{Total number of cells in control and treatment}} \times 100$$

(Sehgal *et al.,* 2006)

$$\text{Frequency of chromosomal aberration(Freq. CA)} = \frac{\text{Total number of abnormal cells in respective treatment}}{\text{Total number of cells counted in respective treatment}} \times 100$$

(Chandrakar *et al.,* 2014)

$$\text{Mean total Percent of abnormal cell(Abn\%)} = \frac{\text{Total number of abnormal cells in respective treatment}}{\text{Total number of dividing cells in control} + \text{Total number of dividing cells in treatment}} \times 100$$

(Bhatta and Sakya, 2008)

$$\text{SDS tolerance(SDS Tol\%)} = \frac{\text{Total number of dividing cells in treatment}}{\text{Total number of dividing cells in control}} \times 100$$

(Asadi *et al.,* 2012)

Chapter – 3

Results and Discussions

Number of meiotic cells in *Allium* species

The mean number of meiotic cells for the *Allium* species were counted and reported in the Table 1. The meiotic cells were counted as the number of dividing cells (DC), number of non dividing cells (NDC) and total number of meiotic cells (TC). The dividing cells were tried to include the meiotic cells which are in active dividing stages of the meiosis and the non dividing cells tried to include meiotic cells without any active meiotic stages during the cell division while addition of both composed of the total meiotic cells.

The mean value of meiotic cells in control for *Allium ascalonicum* was recorded as 40.4±10.9, 760.8±121.0 and 801.2±130.3 for DC, NDC and TC. The dividing cells recorded less as compared to the non dividing cells (Table 1). The treatment with SDS salt concentrations (500ppm, 1000ppm and 1500ppm) has not shown any clear trend of decreasing or increasing order of DC or NDC. But it is obvious that number of meiotic cells (mean±S.E.) were recorded less in treatments at all the concentrations as compared to the control. It was also observed that dividing cells were increasing from lower concentration to the higher concentration although mean value is less than control. It

seems all the concentrations have some inducing effect for the dividing cells but not at par with the control. NDC has not shown any trend of inducing or suppressing effect of the concentrations.

The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance were recorded (SDS Tol %). The mean value of abnormal cells has shown the increasing order from lower concentration to higher concentration (Table 1). It suggested that abnormality in the *Allium ascalonicum* is dose dependent as we increased the concentration abnormality is also increasing. Percent abnormality has shown a similar trend as of mean abnormality of increasing order. Percent abnormality indicated that the applied concentrations are highly toxic to species and has shown more than 60% abnormality at 1000ppm. It also indicates the high sensitivity of the species towards SDS. The frequency of the chromosomal aberration was recorded as 3.38, 5.35 and 5.19 for 500ppm, 1000ppm and 1500ppm respectively. The frequency of chromosomal aberrations increasing from the lower concentrations to the higher concentrations but the frequency of chromosomal aberrations becomes almost constant at the 1000ppm and 1500ppm. The tolerance percent of the SDS salt to meiotic cells is increasing from the lower to higher concentration. It indicates that as we increase the concentration some physiological changes are happening in the cell and to cope up with these changes and at the same time pretend to functions normally meiotic cells increasing its tolerance level. Tolerance level was recorded upto 45.54% at the highest concentration.

The mean value of meiotic cells in control for *Allium hookeri* was recorded as 88.2±34.2, 810.8±271.1 and 899±267.5 for DC, NDC and TC. The dividing cells recorded less as compared to the non dividing cells (Table 1). The treatment with SDS salt concentrations (500ppm, 1000ppm and 1500ppm) has not shown any clear trend of decreasing or

increasing order of DC or NDC. But it is obvious that number of meiotic cells (mean±S.E.) were recorded less in treatments at all the concentrations as compared to the control. It was also observed that dividing cells were increasing from 500ppm concentration to the 1000ppm concentration but suddenly decrease in cells was observed at 1500 ppm, although mean value is less than control. It seems all the concentrations 500ppm and 1000ppm have some inducing effect but 1500ppm has suppressing effect for the dividing cells as compared to control. NDC has shown similar trend of inducing or suppressing effect as in dividing cells. The high number of non-dividing cells at all the concentration indicated that this concentration induced some kind of abnormalities that increases the non-dividing cells.

The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance were recorded (SDS Tol %). The mean value of abnormal cells has shown the increasing order from lower concentration to higher concentration (Table 1). It suggested that abnormality in the *Allium hookeri* is dose dependent as we increased the concentration abnormality is also increasing. Percent abnormality has shown the similar trend as of mean abnormality of increasing order. Percent abnormality indicated that the applied concentrations are toxic and has shown approximately 60% abnormality at highest concentration of 1500ppm. The frequency of the chromosomal aberration was recorded as 2.54, 4.88 and 11.75 for 500ppm, 1000ppm and 1500ppm respectively. The frequency of chromosomal aberrations is increasing from the lower concentrations to the higher concentrations which may suggest the dose dependent effect of SDS concentration and frequency of chromosomal aberrations. The tolerance percent of the SDS salt to meiotic cells was recorded high for 500ppm and 1000 ppm that is 38.77 and 43.76 respectively. But suddenly tolerance level was decreased to 17.23%. It indicates that as

we increase the concentration some physiological changes are happening in the cell and to cope up with these changes and at the same time pretend to functions normally meiotic cells increasing its tolerance level. Tolerance level was recorded upto 43.76% at the 1000 ppm concentration. But at highest concentration the cells gave up to cope up with the concentration and seems most of the cells become aberrant or non functional or pretend to become dead with no tolerance activity towards SDS.

The mean value of meiotic cells in control for *Allium tuberosum* was recorded as 75.8±17.2, 576.8±177.9 and 652.6±179.7 for DC, NDC and TC. The dividing cells recorded less as compared to the non dividing cells (Table 1). The treatment with SDS salt concentrations (500ppm, 1000ppm and 1500ppm) has shown a clear trend of increasing order of DC but not at par with the control cells, on the other hand, no trend was recorded for NDC initially it has shown increased value from 500ppm to 1000ppm but suddenly decreased at 1500ppm. The NDC was recorded more at 1000ppm and 1500ppm than the control. But it is obvious that number of meiotic cells (mean±S.E.) were recorded less in treatments at all the concentrations for dividing cells but not for the NDC and TC as compared to the control. It was also observed that dividing cells were increasing from lower concentration to the higher concentration although mean value is less than control. It seems all the concentrations have some inducing effect for the dividing cells but not at par with the control. NDC has not shown any trend of inducing or suppressing effect of the concentrations.

The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance were recorded (SDS Tol %). The mean value of abnormal cells has shown the increasing order from lower concentration to higher concentration (Table 1). It suggested that abnormality in the *Allium tuberosum* is dose dependent as we increased the

concentration abnormality is also increasing. Percent abnormality has shown the similar trend as of mean abnormality of increasing order. Percent abnormality indicated that the applied concentrations are toxic and has shown more than 62% abnormality at 1500ppm. The frequency of the chromosomal aberration was recorded as 5.13, 5.29 and 9.94 for 500ppm, 1000ppm and 1500ppm respectively. The frequency of chromosomal aberrations increasing from the lower concentrations to the higher concentrations but the frequency of chromosomal aberrations has not shown much difference at the 500ppm and 1000ppm. The tolerance percent of the SDS salt to meiotic cells is increasing from the lower to higher concentration. It indicates that as we increase the concentration some physiological changes are happening in the cell and to cope up with these changes and at the same time pretend to functions normally meiotic cells increasing its tolerance level. Tolerance level was recorded upto 50.39% at the highest concentration.

The mean value of meiotic cells in control for *Allium porrum* was recorded as 94.2±23.1, 571.4±31.1 and 665.6±33 for DC, NDC and TC. The dividing cells recorded less as compared to the non dividing cells (Table 1). The treatment with SDS salt concentrations (500ppm, 1000ppm and 1500ppm) has shown a clear trend of decreasing order of DC but not at par with the control cells, on the other hand, increasing trend was recorded for NDC from lower to higher concentrations and the values were recorded much higher than the control. But it is obvious that number of meiotic cells (mean±S.E.) were recorded less in treatments at all the concentrations for dividing cells but not for the NDC and TC as compared to the control. It was also observed that dividing cells were decreasing from lower concentration to the higher concentration although mean value is less than control. It seems all the concentrations have some inducing effect for the dividing cells as well as non dividing cells.

The number of non-dividing cells was recorded very high at all the concentration and increased from lower concentration to higher concentration. The high number of non-dividing cells at all the concentration suggests that these concentrations are highly toxic to the cells of *A.porrum.* These concentrations are enough to induce physiological cytotoxic or clastogenic aberration in the meiotic cell of *A.porrum*.

The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance (SDS Tol %) were recorded. The mean value of abnormal cells has shown the increasing order from lower concentration to higher concentration (Table 1). It suggested that abnormality in the *Allium porrum* is dose dependent as we increased the concentration abnormality is also increasing. Percent abnormality has shown a similar trend as of mean abnormality of increasing order. Percent abnormality indicated that the applied concentrations are toxic and has shown 43.8% abnormality at 1500ppm. The frequency of the chromosomal aberration was recorded as 1.90, 3.33 and 6.07 for 500ppm, 1000ppm and 1500ppm respectively. The frequency of chromosomal aberrations is increasing from the lower concentrations to the higher concentrations. The tolerance percent of the SDS salt to meiotic cells was recorded high for 500ppm and 1000 ppm that is 25.4 and 24.4 respectively. But suddenly tolerance level was decreased to 14.8%. It indicates that as we increase the concentration some physiological changes happening in the cell and to cope up with these changes and at the same time pretend to functions normally meiotic cells increasing its tolerance level. Tolerance level was recorded upto 24.4% at the 1000ppm concentration. But at highest concentration the cells gave up to cope up with the concentration and seems most of the cells become aberrant or non functional or pretend to become dead with no tolerance activity towards SDS.

The mean value of meiotic cells in control for *Allium chinense* was recorded as 43.8±11.9, 556.6±129.6 and 600.4±127.7 for DC, NDC and TC. The dividing cells recorded less as compared to the non dividing cells (Table 1). The treatment with SDS salt concentrations (500ppm, 1000ppm and 1500ppm) has shown a clear trend of decreasing order of DC but not at par with the control cells, but the values has not shown much differences at the 500ppm, 1000ppmand 1500ppm, on the other hand, no trend was recorded for NDC from lower to higher concentrations and the values were recorded little higher at 500ppm than the control. But it is obvious that number of meiotic cells (mean±S.E.) were recorded less in treatments at all the concentrations for dividing cells as well as NDC and TC except the 500ppm as compared to the control. It was also observed that dividing cells were decreasing from lower concentration to the higher concentration although mean value is less than control. It seems all the concentrations have some suppressing effect for the dividing cells.

The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance (SDS Tol %) were recorded. The mean value of abnormal cells has shown the increasing order from lower concentration to higher concentration (Table 1). It suggested that abnormality in the *Allium chinense* is dose dependent as we increased the concentration abnormality is also increasing. Percent abnormality has shown the similar trend as of mean abnormality of increasing order. Percent abnormality indicated that the applied concentrations are highly toxic and has shown 50.97% abnormality at 1000ppm which increased upto 70.16% at 1500ppm. The frequency of the chromosomal aberration was recorded as 3.57, 18.4 and 21.94 for 500ppm, 1000ppm and 1500ppm respectively. The frequency of chromosomal aberrations is increasing from the lower concentrations to the higher concentrations. The tolerance percent of the SDS salt to meiotic cells was

recorded high for all the concentrations. It indicates that as we increase the concentration some physiological changes happening in the cell and to cope up with these changes and at the same time pretend to functions normally meiotic cells increasing its tolerance level.

Lethal Dose 50(LD_{50}) of different *Allium* species

The Lethal Dose 50 percent (LD_{50}) concentration of SDS on all the *Allium* species (*A. ascalonicum*, *A. hookeri*, *A. tuberosum*, *A. porrum* and *A. chinense*) taken under present study recorded as follows (781.86, 1246.80, 1194.26, 1712.30 and 980.96 ppm) where the 50% of the cells of the species were reported as abnormal or toxic respectively. The highest and lowest concentration (LD_{50}) was recorded for the *A. porrum and A. ascalonicum* respectively (Table 2).

Correlating dose (concentration), mean number meiotic cells and abnormalities among *Allium* species

Pearson correlation of dose (concentration), number of meiotic cells and abnormalities in *A. ascalonicum* showed that number of non dividing cells has significant role in the total number of meiotic cells at $p \leq 0.01$. The increased number of TC are significantly (0.994**) contributed by NDC Pearson correlation (2-tailed) at $p \leq 0.01$ (Table 3).

Pearson correlation of dose (concentration), number of meiotic cells and abnormalities in *A. hookeri* showed that dose applied has significant role in the induction of abnormalities as well as also number of non dividing cells has significant role in the total number of meiotic cells at $p \leq 0.01$. The increased number of abnormalities (0.996**) and TC are significantly (0.999**) contributed by dose dependent response and NDC indicated by Pearson correlation (2-tailed) at $p \leq 0.01$ (Table 4).

Pearson correlation of dose (concentration), number of meiotic cells and abnormalities in *A. tuberosum* showed that number of non-dividing cells has significant role in the total number of meiotic cells at $p \leq 0.05$. The increased number of TC are

significantly (0.989*) contributed by NDC indicated by Pearson correlation (2-tailed) at p≤0.05 (Table 5).

Pearson correlation of dose (concentration), number of meiotic cells and abnormalities in *A. porrum* showed that dose applied has significant role in the induction of abnormalities as well as number of non dividing cells at p≤0.05 and p≤0.01 respectively. The increased number of abnormalities (0.983*) and (0.991**) are significantly contributed by dose dependent response and NDC indicated by Pearson correlation (2-tailed) at p≤0.05 and p≤0.01 respectively (Table 6).

Pearson correlation of dose (concentration), number of meiotic cells and abnormalities in *A. chinense* showed that number of non dividing cells has significant role in the total number of meiotic cells at p≤0.01. The increased number of TC are significantly (1.000**) contributed by NDC indicated by Pearson correlation (2-tailed) at p≤0.01 (Table 7).

Observation on the meiotic stages in different *Allium* species

From the different types of *Allium* species studied, the mean value of meiotic stages was calculated for SDS treatment as well as for Control. It was observed that the mean value of meiotic stages recorded in Control for *A.ascalonicum, A. hookeri*, *A. tuberosum*, *A. porrum* and *A. chinense* was observed to be (40.4±17.9, 88.2±22.2, 75.8±29.6, 94.3±24.5 and 43.8±16.9) respectively (Table No.8). Mean value of meiotic stages was recorded to be maximum at *A.porrum* and minimum at *A.ascalonicum.* Where the maximum mean value of meiotic stage was found to be pachytene (19.8±8.2) in *A.ascalonicum*, TelophaseII (39.8±2.1) in *A.hookeri,* diakinesis (28±14) and TelophaseII (28.8±1) in *A.tuberosum*, Diakinesis (19±21.4, 16.2±4.4) in *A.porrum* and *A.chinense* respectively. However, a gradual decrease in meiotic stages was observed, when SDS treatment was applied for all the *Allium* species.The maximum mean value was recorded to be

Diakinesis(23.8±10.4,19.8±8.5) in *A.ascalonicum* and *A.hookeri* at 1500ppm,1000ppm respectively,followed by AnaphaseI (22.1±20.3),leptotene(17.4±4.5) and Metaphase I(15±11.3) of *A.tuberosum, A.porrum* and *A.chinense* at 500ppm,1500ppm,500ppm respectively,which suggested a dose dependent response of the meiotic activity and concentrations hinders at different levels or points of cell cycle and affect the cell activity as a result few number of meiotic stages were observed as the dose increased (Table 9-13)

Observation on the types of meiotic stages in *Allium* species

On the basis of observation recorded in *Allium* species studied in Control, maximum and minimum mean value was observed where Pachytene meiotic stages was observed to be maximum (19.8±8.2) in *A. ascalonicum*, on the other hand, telophase II (39.8±2.1) for *A. hookeri*, telophase I (28.8±1) and diakinesis (28±14) for *A. tuberosum*, diakinesis (19±12.4) for *A. porrum* and diakinesis (16.2±4.4) for *A. chinense* under control condition respectively. In SDS treatment the maximum mean value of meiotic stage was recorded to be diakinesis (23.8±10.4,19.8±8.5), anaphase I(22.1±20.3), leptotene(17.4±4.5),metaphase I (15±11.3)in *A.ascalonicum*, *A. hookeri, A. tuberosum*, *A.porrum*, *A. chinense* at 1500ppm, 1000ppm, 500ppm, 1500ppm and 500ppm respectively. In the observations on the mean value of the meiotic images of *Allium* species under control and treatment condition, has not shown any specific trend of decreasing or increasing order but the observed results showed the applied concentration to have an effect on the cell cycle and reduced the number of meiotic stages under treatment as compared to the control (Table 9-13).

Observations on the various types of abnormalities in *Allium* species

Observation on various types of abnormalities were recorded in the *Allium* species studied where similar kinds of abnormalities occurred in all the species at different

concentrations (500ppm,1000ppm,1500ppm)of SDS treatment. The abnormalities observed were stickiness, micronuclei, disjunct, fragments, laggards, multivalent formation, strayed chromosomes, loop like structure of chromatin material, loose arrangement of chromatin material, twisted structure of chromosomes, interwoven structure of chromosomes, split or gap among the chromosome, unequal distribution of the chromosome at the poles, univalent formation, bivalent formation, chromosome breaks, chromosome bridges, ring chromosome, nucleomixis, cytomixis, and nucleo-cytomixis. Stickiness was the most common type of abnormality observed for the *Allium* species studied which suggested dose dependent response as it has increased from lower concentration to higher concentration. The mean value of stickiness increased (11±6.8, 19.2±6.5, 20.4±8.4), (5.8±3.3, 8.2±2.7, 10.6±2.9), (11.4±3.3, 20.8±7.6, 37.2±12.3) from lower concentration (500 ppm) to higher concentration (1500 ppm) in *A. ascalonicum*, *A. hookeri*, *A. porrum*, which indicated the dose dependent response while, *A. tuberosum* has not shown any increasing or decreasing trend (13.4±3.6,39.6±11.2, 10.8±1.5), but *A. chinense* showed a slight decreasing trend (16.9±9.4, 14.2±5.2, 13.4±3.4) from the lower to higher concentration. Other abnormalities observed in various *Allium* species were also recorded which includes micronuclei,fragment in metaphaseI and telophase I,cytomixis and unequal development of chromosomes,two overlapping interphase cells,bridges and disjunction at anaphaseI all observed in *A.ascalonicum*. Micronuclei, non-dividing cells, no proper separation of chromosomes at diakinesis, two overlapping cells at interphase are also types of abnormality observed in *A.hookeri.*Restitution nucleus at telophase I,bridge and disjunction in anaphase I,uncondensed chromatin ,overlapping of cells ,micronuclei in chromatin nucleus all observed in *A.tuberosum.* Some abnormalities such as binucleate cells with scattered chromosones, disturbed zygotene were observed in

A.porrum. In *A.chinense,* abnormality observed were stickiness in interphase stage with presence of microspore also being reported (Figure Plate No.1-11).

Correlating dose (concentration) and types of abnormalities in *Allium* species

All the *Allium* species were correlated for their different dose treatment (500ppm, 1000ppm, 1500ppm) and types of abnormalities produced and tried to find the association or degree of dose effect and abnormalities induced in the species (Table 19-33).

Allium ascalonicum

To observe the association of different types of abnormality produce at all the concentration, correlation was done and we observed that stickiness of the chromatin material was associated with almost all different type of abnormality produced in the species significantly or insignificantly at $p \leq 0.01$ and $p \leq 0.05$. Sticky chromatin material maybe involved significantly in the formation of laggards (0.954*), disjunction (0.954*), star shape (0.988**), ring structure of chromosomes (0.988**), unequal distribution of chromosomes (0.988**) at $p \leq 0.01$ and $p \leq 0.05$.The association of different types of chromosomes clearly indicates that they are derive from stickiness structure of material in the species at 500ppm. Similarly, laggards may be involve in the formation of disjunction (1.000**), star shape structure (0.943*), ring chromosomes (0.943*) and unequal distribution of chromosomes (0.943*) at $p \leq 0.01$ and $p \leq 0.05$.The disjunction chromosomes may also produce from the star shape structure of chromosomes (0.943*), ring structure of chromosomes (0.943*) and unequal distribution of chromosomes (0.943*) at $p \leq 0.05$. Star shape structure of chromosomes has participated 100 percent for the formation of ring structure (1.000**) and unequal distribution of chromosomes (1.000**) at $p \leq 0.01$. Ring chromosome may also be observed when there is an unequal distribution of chromosomes (1.000**) $p \leq 0.01$ (Table 19).

The application of 1000ppm of SDS concentration in *A. ascalonicum* produce less number and types of abnormal cells as compared to lower and high concentrations. The abnormality produce at 1000ppm has not shown any significant relation with other type of abnormality and concentration. The production of laggards has shown significant relation with sticky nature of chromosome material and laggard (0.964*) are derived from sticky nature of the genetic material in a meiotic cell at $p \leq 0.01$ (Table 20).

The application of 1500 ppm in *A. ascalonicum* produces different numbers and types of abnormality which are associated with each other significant or insignificantly. Bridges has shown significant association with cytomixis (0.968**) at $p \leq 0.01$ i.e., during the formation of bridges may induce some chromosomes to move into different directions to form a multipolar structure of cell. The production of multipolarity may involve in the formation of nucleomixis (0.887*) at $p \leq 0.05$.Chromatin gaps has shown 100 percent association with the chromosomes fragments (1.000**) at $p \leq 0.01$clearly indicates that when the formation of chromatin gaps takes place simultaneously there will be chromosome fragments which give this type of abnormalities. It suggests the formation of fragments chromatin material parts from them and leaves a gap among the chromatin materials (Table 21).

Allium hookeri

The meiotic cell abnormality was correlated for their association of different types of abnormality in response to different doses. At 500ppm, stickiness abnormality leads to different kinds of abnormality such as laggards (0.971**), unequal distribution of chromosomes (0.971**), nucleomixis (0.985**), cytomixis (0.971**), star shape

structure (0.971**) and fragment (0.971**at p≤0.01). Similarly, laggards which was induced by stickiness participated in the formation of unequal distribution of chromosomes (1.000**), nucleomixis (0.996**), cytomixis (1.000**), star shape (1.000**) and fragments (1.000**). It seems that laggards were involved 100percent in the induction of abnormalities (unequal distribution of chromosomes, nucleomixis, cytomixis, star shape, fragment).Unequal distribution of chromosomes has shown similar trend as laggards which involve in the induction of nucleomixis (0.996**), cytomixis (1.000**), star shape (1.000**) and fragments (1.000**). Nucleomixis has also contributed to produce cytomixis (0.996**), star shape (0.996**) and fragments (0.996**) at p≤0.01chromosomal activities. Cytomixis has shared 100percent for the induction of star shape (1.000**) and fragments (1.000**) of the chromosomes. Star shape structure also induce fragments (1.000**).The induction and correlation of the abnormality at 500ppm suggest that these abnormalities are interdependent and derived from one abnormality to another abnormalities (Table 22).Stickiness among the chromosomes was the higher abnormality from where all other types of abnormalities induced which are physiological in nature. All the abnormalities at 500ppm do not affect the chromosome but they affect the chromosomal protein which leads to these types of abnormalities.

At 1000ppm, the correlation of different abnormalities in 1000ppm suggested that stickiness is not involved in the production of the abnormalities. It suggests that the concentration has affected the chromosomal protein and induce independent instead of derived from sticky chromosomes. Correlation between multipolarity and laggards (-0.896*) at p≤0.05 has shown a negative correlation which means there is no relation or production of laggards during the formation of abnormal multipolarity and laggards maybe produced because of stickiness of the chromosomes. The formation of

chromosomal bridges may be involve in the formation of unequal distribution of chromosomes (0.904*) at p ≤0.05.The formation of micronuclei may be the result of unequal distribution of chromosomes (0.897*) or the production of fragments (0.958*) at p≤0.05 during the application of SDS concentration. The formation of split chromosomes may take place during the star shape structure (1.000**) form of chromosomes after application of SDS concentration. It also indicated that star shape structure formation contributed 100 percent for the formation of split chromosomes after application of the concentration (Table 23).

The application of 1500ppm of SDS indicates that different abnormalities are produce by the action of these concentrations on the proteins as well as chromosomes structure. The abnormalities were not derived from the sticky chromosomes as it has not shown any significant correlation with produced abnormalities. The formation of bridge aberrations leads to the formation of laggards (0.980**) at p≤0.01.The formation of micronuclei observed shows direct relationship with formation of chromosomal ring (0.907*) at p≤0.05.It also suggest that micronuclei may form after a small break from the chromosomal arm and the breakage in the chromosomes leads to the formation of ring structure of the chromosomes by sticking two ends of the chromosomal arms. The disjunction of chromosomes resulted from the multipolar distribution (1.000**), nucleomixis (0.943*), cytomixis (0.968**) at p≤0.01and formation of 3 micronuclei (1.000**) cells at p≤05 and p≤.01. Multipolar chromosomes may form during the nucleomixis (0.943*) or cytokinesis (0.968**) and also involve in the production of three micronucleate cells (1.000**) at p≤0.05 and p≤0.01.The ring formation of chromosomal abnormalities may lead to the formation of chromosomal gaps (0.917*) at p≤0.05. Star shape structure of chromosomes may bring irregular structure of chromosomes (0.943*) at p≤0.05. The nucleomixes type of abnormality directly related to the abnormality

induce during the cytomixis (0.996**) and the formation of three micronucleate cells (0.943*) at p≤.01 and p≤.0.05.Cytomixis abnormality induce the three micronucleate cells (0.968**) at p≤0.01. At lower concentration, it acts on the physiology of the cells (Table 24).

Allium tuberosum

The association of different types of abnormalities has shown no significant correlation for the 500ppm dose application to the *A.tuberosum* except stickiness (0.917*) and unequal distribution of chromatin material (0.866*) at p≤0.05. Sticky chromatin material leads to the formation of unequal distribution of chromosomes and during this process some chromosomes lagged behind and unable to reach to the poles and acts as laggards (Table 25).

Stickiness abnormality was involved in the formation of unequal distribution of chromosomes at p≤0.05, but when 1000ppm dose was applied, the different types of abnormalities are significantly correlated with each other. Stickiness of the chromosomes has shown maximum but the association of sticky chromosomes was not significant with other different types of abnormality produce at 1000ppm in *A.tuberosum*. It also suggest that the concentration has effect in different cells and produce different types of abnormality separately without derivation of the type of abnormality from the sticky nature of chromatin material .The formation of micronuclei highly associated with macronuclei (0.991**) and nucleomixis (0.985**) at p≤0.01. Micronuclei as a result of breakage in chromatin material and the breakage part surround themselves to form a nucleus type genetic material which is call micronuclei and this similar size nuclei infuse together to form a bigger size nucleus called macronuclei and the formation of macronuclei may occur during the nucleomixis abnormality where two nucleus fuse together to form a nucleomixi type abnormality. During the nucleomixis there is a

probability in the formation of smaller size and bigger size nucleus. Macronuclei is significantly associated with nucleomixis type (0.970**) at p≤0.01 of abnormality. Macronuclei may form when two different nucleus are mixed together and some genetic material left behind to take a shape of macronuclei. The fragment are associated 100percent with laggards abnormality(1.000**) at p≤0.01, a fragment are produce by the act of SDS concentration and this fragment remain in the cell in the form of laggards which pass from one cell to another cell or may disappear after few cell cycles. Ring structure of chromosomes are highly associated with unequal distribution of chromatin material (0.993**), multipolar (1.000**), strayed formation of chromatin material (1.000**) at p≤0.01. It clearly indicates that the ring structure chromosomes are form when there is a physiological abnormality in the cell and chromatin material abnormally distributed towards the unequal multipolarity or strayed chromosomes which leads to the formation of ring chromosomes where the terminal region of some chromosomes become sticky and attached together to form a chain or ring structure of chromatin material. Similarly, unequal distribution of chromosomes are highly correlated with multipolar formation (0.993**), strayed formation (0.993**) and disjunction chromosomes (0.993**) at p≤0.01. Similar multipolar formation also involve in the formation of strayed chromatin (1.000**) and disjunction chromatin material (1.000**).The strayed chromosome directly associate with the formation of disjunction chromatin material (1.000*) at p≤0.01.The abnormality produced at 1000ppm are mostly physiological in *A.tuberosum* except the formation of micronuclei, macronuclei and ring structure chromosomes which suggest the clastogenic nature of the concentration for the species (Table 26).

The application of 1500ppm has produce less number of different type of abnormality in which some abnormality has shown negative association but not significance. The

significant association was observed between the laggards and the chromatin gaps (1.000**) at p≤0.01 and ring structure of chromosomes with nucleomixis (0.925*) at p≤0.05. The formation of laggards by the application of 1500ppm concentration results in the chromatin gaps which produce gaps among the chromatin material and acts as laggards.Similarly the ring structure chromosomes were formed when the two nucleus are mixed together by the physiological abnormality of meiotic cell (Table 27).

Allium porrum

The production of ring types of chromosomes may involve in the formation of micronuclei (1.000**) viz versa after application of 500ppm concentration at p≤0.01 (Table 28). The application of 1000ppm in *A.porrum* has not shown any significant abnormality and the production of abnormality were less than 500ppm and 1500ppm (Table 29). The application of 1500ppm has shown that production of fragments may involve in formation of ring types of chromosomes (0.932*) at p≤0.05 (Table 30). The production of ring chromosome and micronuclei at 500ppm indicates the clastogenic nature of these concentrations which directly affect the genetic material of the cell than the proteins involve in the physiological activity of the cell.

Allium chinense

The abnormality produced was correlated with each other at all the concentration to observe their association and their production after application of different concentration. In 500ppm, it was observed to be the most effective and produced maximum number of abnormality where, the stickiness has shown its association with other different types of abnormality produced significantly at p≤0.05 and p≤0.0.1.The stickiness among the chromosomes has shown its association for the production of laggards (0.890*), disjunction (0.904*), multipolarity (0.890*), strayed chromosomes (0.904*), loose chromatin material (0.904*), unequal distribution of chromosomes(0.904*) at p≤0.05 and

the production of gaps (0.981**) among the material at p≤0.01.The micronuclei may be induced from the sticky chromosomes by the physiological activity of cells and by the effect of the applied concentrations and produced micronuclei maybe involve in the formation of other types of abnormality such as fragments(0.980**), laggards (0.968**), multipolarity (0.968**), abnormal structure of chromatin material (1.000**) at p≤0.01 and loop formation(0.949*), intermingled chromatin material (0.949*) and transition of chromatin material (0.919*) at p≤0.05. The fragment of chromatin material may be produce from the lagging chromosomes or laggards chromatin material (0.900*), multipolarity chromosomes (0.900*), intermingled chromosomes (0.937*), univalent (0.896*), bivalent (0.896*), chromatin breaks (0.896*), ring chromosomes (0.896*), bridges (0.896*) and translocation zone (0.896*) at p≤0.05). It can also be produce from loop formation (0.993**), chromosomes twisted (0.980**) and transition chromosomes (0.966**) at p≤0.01.The production of laggards was observed from multipolarity (1.000**) and twisted chromosomes (0.968**) at p≤0.01. The intermingling (0.910*) of chromatin material also involve in the formation of laggards at p≤0.05. The disjunction of chromosomes may arise from strayed type of chromosomes (1.000**), loose chromosomes (1.000**), unequal distribution of chromosomes (1.000**) or from the gap among chromosomes at (0.968**) p≤0.01. The multipolar chromosomes may arise from the twisted (0.968**) type of chromosomes or in the intermingling (0.910*) of chromosomes at p≤0.01 and p≤0.05.The production of strayed chromosomes were observed from loose type of chromosome (1.000**), unequal distribution of chromosomes (1.000**) and gaps (0.968**) among the chromosomes at p≤0.01.The formation of loops occur due to the involvement or may involve other different type of abnormality such as twisted (0.949*), intermingling (0.912*), formation of univalent (0.943*), bivalent (0.943*), chromatin breaks (0.943*), ring chromosomes (0.943*),

bridges (0.943*) and transition zone (0.943*) at p≤0.05 and p≤ 0.01. The gaps (0.968**) and unequal distribution of chromosomes (1.000**) involve in the formation of loose type of chromatin material at p≤0.01. The abnormal structure of chromatin material or twisted chromosome material may arise from the intermingling (0.949*) and transition (0.919*) of the chromatin material at p≤0.05. The transition of chromatin material or paring of chromatin material forming a chiasma or crossing over of material may lead to the formation of bridges. Ring chromosomes, breaks, bivalent and univalent of chromosomes at p≤0.01.The unequal distribution of chromatin material produce due to the present of small gaps among the chromatin material which leads to distribution of unequal chromatin material (0.968**) at both the poles at p≤0.01. Univalent chromosomes were found to be 100percent correlated with bivalent (1.000**), chromatin breaks (1.000**), ring chromosomes (1.000**), bridges (1.000**) and translocation (1.000**) at p≤0.01. Bivalent chromosomes were also found to be 100 percent correlated with chromatin breaks (1.000**), ring chromosomes (1.000**), bridges (1.000**) and translocation (1.000**) at p≤0.01.Chromatin breaks 100percent correlated with ring chromosomes (1.000**), bridges (1.000**) and translocation (1.000**) at p≤0.01. Ring structured chromosomes 100 percent correlated with bridges (1.000**) and translocation (1.000**) at p≤0.01. Bridge chromosomes 100 percent correlated with translocation (1.000**) at p≤0.01 (Table 31).

The application of 1000ppm in *A.chinense* has produced many abnormalities but the produced abnormality is less in number compared to the 500ppm where the maximum number of abnormality is observed. The produced abnormalities are correlated among themselves for their association and involvement for the production of other different type of abnormality at 1000ppm. The sticky chromosome has not shown any significant association for the production of other type of abnormality but the micronuclei may be

induce by the intermingling or unequal distribution of chromosomes and formation of bridge which had shown 100 percent association with these abnormalities at p≤0.01. The production of disjunction and cytomixis abnormality also involves in the formation of fragment and show 100 percent association of these abnormality of forming fragment at p≤0.01.During the mixing of cytoplast some chromosomes may move apart from the other chromosomes and makes a disjunction type of chromosomes at p≤0.01.Cytomixis has shown 100 percent association for the formation of a disjunct type of chromosomes. The intermingling of the chromatin material may involve in the formation of unequal distribution of the chromosomes at two different poles and also the formation of bridges where two chromosomes are stick together to form a long bridges between two poles at p≤0.01.unequal distribution also share 100percent association in the formation of chromatin bridges at p≤0.01. The 100 percent association of abnormalities suggest that the concentration applied is highly effective in the production of different abnormality and may be meiotic cells of *A.chinense* sensitive to these applied concentration which affect the genetic material and causes genotoxicity. The genotoxic activity may due to clastogenic or physiological effect of this concentration on the genetic material (Table 32).

The application of 1500ppm in *A.chinense* produces many abnormalities and stickiness abnormality has not shown any significant correlation with the formation of other different types of abnormalities. The productions of micronuclei occurs during the formation of multipolar chromosomes (1.000**) at p≤0.01. The nucleomixis (-0.978**) has shown significant and negative correlation with the production of fragment at p≤0.01.The negative correlation indicates that the nuclear mix does not involve in the formation of production of any genetic material. It also indicates that it has a mixing of two genetic materials after dissolving the nuclear membrane when a concentration

(1500ppm) was applied. It showed the effect of 1500ppm on the nuclear membrane of a nucleus and also showed its high genotoxicity towards the nuclear material. The mixing of cytoplast (-0.885*) also shown negative and significant correlation at $p \leq 0.05$ which indicates that mixing of cytoplast do not involve in the formation of disjunction at two different poles. The abnormality such as nucleomixis, cytomixis, unequal distribution of chromosomes and multipolarity may be involved in the formation of univalency, bivalency and ring type of chromosome abnormalities at $p \leq 0.01$. The concentrations 500ppm, 1000ppm, 1500ppm in *A.chinese* has shown clastogenic nature of activity as it produce micronuclei and ring chromosomes or moreover, it has activity in the genetic material which lead to other abnormalities such ring chromosomes and micronuclei which control genetic information and may or may not be release into the next cell division cycle, if not release than it produces abnormality which remain in the cell cycle and pass from one cell to another cell so these abnormality may be check before entering into the next division cell cycle by the check points of the cell cycle at each transition stage during the cell division cycle. We observed maximum stickiness but it has not shown any significant relationship with other abnormalities, which suggest that, the concentration 500ppm, 1000ppm, 1500ppm has direct effect on the genetic material than the protein involve in the cell division cycle. The direct affect of these concentrations may lead to the genotoxicity, morphological abnormality or mutations in the species, if the concentrations are continuously applied for the study of cell division abnormalities (Table 33).

Physiological types of abnormalities in *Allium* species

The physiological aberrations induce by different concentrations of SDS in different species of *Allium* recorded (Tables 34). The physiological aberrations observed in different *Allium* species at different concentration in the form of laggards, stickiness,

strayed chromosomes, abnormal anaphase and abnormal metaphase. The stickiness physiological aberrations has shown increasing trend from lower concentration to higher concentration in *A. porrum, A. hookeri* and *A. ascalonicum* (approximately which indicates the very close values at 1000 and 1500 ppm), while *Allium tuberosum* could not locate any increasing or decreasing trend of this abnormality on the other hand, *Allium chinense* showed little decreasing trend from lower to higher concentration. The abnormality might be the effect of the chemical or the protein of chromosomes which helps in gluing the different chromosomal arms. The chemical affects the gluing properties of these proteins and leads to the increase stickiness aberrations among the chromosomes. Also the separin protein molecule which helps in separating the two strands of DNA affect may become less functional and increases the stickiness among the chromosomes. The stickiness type of physiological aberrations leads to the production of different other type of physiological aberrations such as laggards, fragments bridge formation, strayed chromosomes, abnormal anaphase unequal distribution of chromosomes and abnormal metaphase stage. Laggards aberration were observed maximum in *Allium ascalonicum* which also suggest that chromosomes are much affected by the chemical and the chromosomes are unable to move towards the pole because of the maximum stickiness observed in the species. The other physiological aberrations such as strayed chromosomes, abnormal anaphase and abnormal metaphase have not shown an increasing or decreasing trend for the different concentration for the species. From the present data it has been observed that stickiness among *Allium* species was maximum and it seem a common type of abnormality occurs in different *Allium* species studied (Table 34).

Clastogenic types of abnormalities in *Allium* species

On the other hand the maximum stickiness of the chromosome in physiological aberrations induced may involve in the induction of clastogenic type of aberrations in the chromosomes of the species. The clastogenic type of abnormality occurs when stress affects the genetic material of the chromosomes of plant or animal species. The different types of clastogenic abnormality were observed such as chromosome breaks, ring chromosome and micronuclei at different concentrations. The chromosomes breaks was increase from lower to higher concentration in *Allium ascalonicum* while *Allium porrum*, *Allium tuberosum* and *Allium chinense* less affected than *Allium hookeri* .The increase number of chromosomal breaks *Allium ascalonicum* suggest that the chemical is highly toxic or not suitable as compared to *Allium porrum, Allium hookeri, Allium chinense* and *Allium tuberosum.* It may suggest that the chemical may not be used for any kind of trend in the *Allium ascalonicum* as it has chromosomal break clastogenic aberrations and there may be a chance to lose the genetic fragment or chromosome fragment during cell division which cannot be recovered back again. Similarly the ring chromosome clastogenic aberrations were observed in increasing trend from lower to higher concentration in *Allium tuberosum*. The highest concentration (1500 ppm) is highly toxic for the species as it induces maximum number of ring chromosomes. But the ring chromosomes may be utilised in both ways as disadvantage and advantages type. The advantage type include that the concentration may be used to induce the chromosomal exchange with non-homologous pair. The disadvantage of these ring chromosomes is that the chromosomes translocate among themselves and increase the homozygous which reduces the plant vigour. The clastogenic aberration form of micronuclei has shown increasing trend in the *Allium hookeri* from lower to higher concentration followed by *Allium ascalonicum* and *Allium chinense.* It seems that all the concentrations of SDS are highly toxic for the species. In the form of micronuclei aberrations, micronuclei may be

induce from the chromosomal breaks and in the successive cell division the break may remain in the cell in the form of nuclei. The fate of the nuclei may remain in the cell for little successive generation or produce different type of abnormality after binding with normal chromosomes or lost from the cell after successive cell division cycle which interferes in the normal cell cycle causing different types of mutations or abnormalities (Table 35).

Meiotic indices (%) in *Allium* species

The highest meiotic index (14.15) was recorded in *A. porrum* followed by the *A. tuberosum* (11.61), *A. hookeri* (9.81), *A. chinense* (7.29) and *A. ascalonicum* (5.04) for control respectively. It clearly indicates that the meiotic cells are highly active in the *A. porrum* as compared to the other species. Meiotic index was recorded less in all the treatments for all the species than control. It is observed that none of the species has shown a sharp and clear cut decrease or increase in index or it may not be related as dose dependent response. The decrease meiotic index (MI) than control suggests the cytotoxicity and genotoxicity of the applied concentration of SDS. When the meiotic index decreases at higher concentration, it also indicates that the total percent of abnormal cell must increase from lower to higher concentration and the total percent of abnormal cell was increase from lower to higher concentration in all the species, where the meiotic index and percent of abnormal cell observed correlates with each other (Table 36).

Observation on sensitivity and resistivity of *Allium* species

On the basis of LD_{50} value, *A. ascalonicum* has recorded the minimum value of 781.86 ppm suggests its sensitivity towards the SDS concentration, followed by *A. chinense, A. tuberosum, A. hookeri* and *A. porrum. A. ascalonicum* requires very less concentration to destroy the cell viability and its physiological activity as well as induction of more

abnormal cells, on the other hand *A. porrum* is little resisting against the SDS concentrations.

On the basis of frequency of chromosomal aberrations, *A. chinense* is maximum sensitive to SDS which at highest concentration affected most of its genome and inducing aberrations at the rate of 21.94% followed by *A. hookeri, A. tuberosum, A. porrum and A.ascalonicum* respectively. *A. porrum* and *A. ascalonicum* suggest little resistance towards the SDS in terms of chromosomal aberration frequency.

On the basis of SDS tolerance, all the species were able to tolerate the concentrations at the highest level, but less tolerance level was recorded in *A.hookeri* followed by *A. porrum*. In this case, *A. hookeri* may be the sensitive and *A. tuberosum* is resisting to the SDS.

On the basis of meiotic index, *A. ascalonicum* and the *A. porrum* may be the sensitive towards the SDS concentrations as they have less MI% in the different concentrations as compared to the other species.

The sensitivity and resistivity of the *Allium* species towards the SDS has predicted a mixed response, it is very difficult to predict which species is the most sensitive and which one is most resistance towards the SDS. The conducted experiment is not enough to predict anything and therefore, it is necessary to conduct more and more experiments in controlled manner.

From the above, it may be derived that applied concentrations of SDS is harmful or toxic to the *Allium* species at cytological, physiological, or genetic level. The various results obtained in the present study are in similar line obtained by the various authors in different organisms and chemicals tested such as chromosomal abnormalities such as stickiness, which may result from breakage and exchange between chromatin fibres on the surface of adjoining chromosomes (Laemmli, 1970; Matta *et al.,* 1981; Shehata *et al.,*

2008; Fisun and Goc Rasgele 2009). On the other hand, disturbed abnormality observed may result from treatment of chemicals on the protein which constitute the spindle apparatus (Polit *et al.,* 2000; Maslam, 2004; Usciati, *et al.,* 2004). Laggards observed in stages of meiotic division can be attributed to the malfunction of spindle apparatus to organize and function in a normal way (Atef *et al.,* 2011). Random distribution of laggards at anaphase, telophase I and telophase II may ultimately results in aneuploidy (Amer and Ali, 1988) or result in micronuclei at telophase (Ozturk 2008). Laggard chromosomes can also be attributed to irregular orientation of chromosomes (Patil and Bhat, 1992). Apart from common abnormalities, other aberrations were observed on meiotic division which includes bridges, micronuclei and multinucleate. The induction of Bridges observed may be due to breakage and reunion (Asita and Makhalemele, 2009) or due to chromosome stickiness (Ozturk, 2008). Micronuclei and multinucleate were also recorded.

Chromosome stickiness is a character of an intense chromosome cluster during cell cycle (Rao *et al.,* 1990). In some cases the abnormal chromosome separation caused bridge and bridge fragmentations. The chromosome fragments formed into micronuclei. In several genera chromosome stickiness has already been reported (Mendes-bonato *et al.,* 2001). For the first time, chromosome stickiness in maize was reported (Beadle, 1932) and ascribed such irregularity to a mutation by a recessive gene called sticky (st). Different species of the genus *Brachiaria* have been published (Mendes-bonato *et al.,* 2001; Utsunomiya *et al.,* 2005; Risso-pascotto *et al.,* 2006; Mendes-bonato *et al.,* 2007; Pagliarini *et al.,* 2008; Risso-pascotto *et al.,* 2009). They propose that chromosome stickiness may be under genetic control, or it may be controlled by a single pair of genes, two pairs of genes or by the interaction of several genes which may be recessive or dominant. Environmental factors such as X-rays, temperature and soil elements may

cause stickiness (Mendes-Bonato *et al.,* 2001). Since they found irregularity in only one, it is suggested that chromosome stickiness is a phenomenon under genetic control. The presence of Aluminum (Al+3) in the soil, besides genetic factor, showed strong evidence that may have caused chromosome stickiness in maize (Caetano-Pereira *et al.*, 1998). According to Rao *et al.* (1990), although chromosome stickiness a cytological phenomenon has been reported for a long time, it is not completely understood. The primary cause and biochemical base of chromosome stickiness abnormality is largely unknown in spite of many factors been suggested (Mendes-Bonato *et al.*, 2007; Gaulden, 1987). It has also been hypothesized that chromosome stickiness may be caused due to a failure or malfunctioning in one or two types of non-histonic chromosome proteins (Dowd *et al.*,1986). According to the number of chromosomes involved in the genome, stickiness has been classified from moderate to severe (Dowd *et al.,*1986). The irregularity has been generally related with chromosome breakage and acentric fragments of different sizes, which may remain as micronuclei in the cytoplasm. Pollen grains produced by cells with chromosome stickiness are generally inviable. The fertilization is jeopardized due to the genetic imbalance caused by the chromosome irregular segregation (Caetano-Pereira *et al.,* 1998).

Bridges which causes structural chromosomes mutation results from chromosome or chromatid, breakage and fusion, which could take place during the translocation of an unequal chromatids exchange or due to presence of dicentric chromosome (El-Ghamery *et al.,* 2000).

Stickiness considered to be a chromatid type aberration have also been attributed to the effect of environmental pollutants on degradation or deploymerization of chromosomal DNA (Darlington and Mc Leish,1951) on DNA condensation (Osterberg *et al.,*1984) and entanglement between chromosomes interactions (Chauhan *et al.*,1986; Goujon *et*

al.,2015). They are a common sign of high toxicity effects on chromosomes and are irreversible that probably leads to cell death (Fiskesjo and Hereditas,1985; El-Ghamery *et al.,*2000). Sub-chromatid linkage between chromosomes can also be the cause of stickiness (Ajay and Sarbhoy,1988) or inability of chromosomes to move and get stuck and failed to reach their final destination (Mc-Gill,1974).Which can also be explained as physical adhesion of the chromosome proteins (Goujon *et al.,* 2015). Laggard Chromosomes are due to spindle failure (Mesi and Kopliku, 2013) and they increase the risk for aneuploidy (Grover and Kaur, 1999; Mesi *et al.,* 2013).

The observation of chromosome breaks showed the clastogenic effect of SDS, in addition to the chromosome fragments, sticky metaphase and polar deviations (wrong directions of chromosome movement). Chromosome stickiness has been attributed to the effect of pollutants and chemical compounds on the physical-chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intra chromatid cross links (Shahin and El-Amoodi, 1991; Rencüzogulları *et al.,*2001; El-Ghamery *et al.,*2003; Gömürgen, 2005; Turkoglu, 2007). The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase and telophase bridges (Sharma and Sen, 2002; Singh, 2003). The induction of chromosome breaks, disturbances on microtubule assembly and cellular death can be related. The frequency of cells with micronuclei is a good indicator of the cytogenetic effects of tested chemicals. Micronuclei(Mnc) often results from the acentric fragments or lagging chromosomes that fail to incorporate into the daughter nuclei during telophase of the mitotic cells and can cause cellular death due to the deletion of primary genes (Albertini *et al.,*2000; Krishna and Hayashi, 2000).

The occurrence of binucleated cells is the result of prevention of cytokinesis or cell plate formation. These results are in accordance with the literature data. Similar inhibition of cytokinesis cells were also reported by Kaushik, (1996), Borah and Talukdar, (2002) and Gomurgen *et al.*(2005). Binucleate cells arise as a consequence of the inhibition of cell plate formation. These form a distinct sub-population of easily detected cells. Failure of cell plate formation in already binucleate cells may give rise to the multinucleate condition. Mitotic irregularities, such as incompleted anaphases or unequal distribution of the chromosomes to the daughter cells can result in aneuploid or even euploid cells. Several pesticides are known to induce the binucleate and multinucleate conditions including bromacil (Ashton *et al.,* 1969),Carbaryl (Amer and Farah,1968), dinoseb (Sawamura,1965), hexa- chlorocyclohexane (Gimenez-Martin *et al.,*1966; Gonzalez,1967; Baquar and Khan,1971), nitralin (Gentner and Burk,1968) and pro-pham (Doxey,1949; Ennis,1948; Canvin and Friesen,1959).

According to Klasterska *et al*. (1976) and McGill *et al*. (1974) it was suggested that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and chromosomes. As a result there is an intermingling of the fibers, and the chromosomes become attached to each other by means of sub-chromatid bridges. Chromatin fibers which join two sister chromatids at metaphase and presumably hold the chromatids together until anaphase have been termed interchromatid connections (Du Praw, 1970). Chromosome fragmentation results from multiple breaks of the chromosome in which there is a loss of chromosome integrity. Fragmentation can range from partial to total disintegration of the chromosome (the latter is termed chromosome pulverization). Chromosome breakage observed is now generally considered to involve the DNA molecule responsible for the linear continuity of the chromosome. Such aberrations are the result of unfinished repair or disrepair of DNA (Evans, 1977).

According to Odeigah *et al.* (1997), sticky chromosomes are indicative of a highly toxic usually irreversible effect, leading to cell death. Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition periods, causing DNA-DNA or DNA-protein cross linking (Amin, 2002). Sticky chromosome might also be the result of incomplete replication of chromosomes by defective enzymes (Bennet, 1977).

Chromosomal abnormalities induced from SDS treatments points to the mutagenic potential of this chemical. The observed chromosomal aberrations in the treated meiotic cells were definitely induced by SDS concentrations since such aberrations were not observed in the control. This suggests that SDS Could be genotoxic at the chromosomal level. The observation of laggards, chromosomes fragments, anaphase bridge and scattering of chromosomes at anaphase is an indication of the capability of SDS in *Allium* species in causing chromosome breakage, resulting in genetic imbalance in the genome. However the type of chromosomal aberration varied. Sticky chromosomes were most frequent in all concentrations.

Chapter - 4

Summary and Conclusion

The *Allium* species (*A. ascalonicum, A. hookeri, A. tuberosum, A. porrum* and *A. chinense*) were collected from different locations of Nagaland and slides were prepared for control as well as treatment of SDS concentration (500ppm,1000ppm,1500ppm) were observed under the microscope and recorded the data on number of meiotic cells, LD_{50}, meiotic images, types of meiotic images, types of abnormalities, physiological, clastogenic, meiotic index, sensitivity and resistivity of different *Allium* species against the SDS concentrations.

The mean value of meiotic cells in control for *Allium ascalonicum*, *Allium hookeri*, *Allium tuberosum*, *Allium porrum*, and *Allium chinense* was recorded as (40.4±10.9, 760.8±121.0 and 801.2±130.3), (88.2±34.2, 810.8±271.1 and 899.0±267.5), (75.8±17.2, 576.8±177.9 and 652.6±179.7), (94.2±23.1, 571.4±31.1 and 665.6±33), and (43.8±11.9, 556.6±129.6 and 600.4±127.7) for DC, NDC and TC respectively. In all the species, the dividing cells were recorded less as compared to the non dividing cells and have shown mixed response. The other parameters such as mean value of abnormal cells (Abn_{Mean}), percent of abnormal cells (Abn %), frequency of chromosomal aberrations (FreqCA) and SDS salt tolerance were recorded (SDS Tol%). All the parameters have shown in increasing order from lower concentration to higher concentration which is dose dependent response for all the *Allium* species.

The Lethal Dose 50 percent (LD_{50}) concentration of SDS on all the *Allium* species recorded as follows (781.86, 1246.80, 1194.26, 1712.30 and 980.96 ppm) where the 50% of the cells of the species were reported as abnormal or toxic respectively. The highest and lowest concentration (LD_{50}) was recorded for the *A.porrum and A.ascalonicum* respectively.

Pearson correlation of dose, number of meiotic cells and abnormalities correlated well to indicate their internation and induction of various types of abnormalities and less dividing meiotic cells at $p \leq 0.05$ and $p \leq 0.01$.

The various meiotic images (mean value) were recorded and observed along with different types of abnormalities such as stickiness, micronuclei, disjunct, fragments, laggards, multivalent formation, strayed chromosomes, loop like structure of chromatin material, loose arrangement of chromatin material, twisted structure of chromosomes, interwoven structure of chromosomes, split or gap among the chromosome, unequal distribution of the chromosome at the poles, univalent formation, bivalent formation, chromosome breaks, chromosome bridges, ring chromosome, nucleomixis, cytomixis, and nucleo-cytomixis in all the *Allium* species for all the concentrations. In all the abnormalities observed, stickiness was found to be the most common type of abnormality for all the *Allium* species, which could be a cause leading to several other abnormalties observed.

Various abnormalities induced in treatments were also correlated and exhibited well connected and associated with each other for further production of other abnormalities for all *Allium* and concentrations at $p \leq 0.05$ and $p \leq 0.01$.

The abnormalities induced were differentiated into physiological and clastogenic types for all the concentrations and *Allium* species. The physiological aberrations observed in the form of laggards, stickiness, strayed chromosomes, abnormal anaphase

and abnormal metaphase and clastogenic abnormality as chromosome breaks, ring chromosome and micronuclei,which induce disruption or breakages of chromosomes..

The highest meiotic index (14.15) was recorded in *A. porrum* followed by *A. tuberosum* (11.61), *A. hookeri* (9.81), *A. chinense* (7.29) and *A. ascalonicum* (5.04) for control respectively. It is observed that none of the species has shown a sharp and clear cut decrease or increase in index or it may not be related as dose dependent response.

Prediction of the sensitivity and resistivity of *Allium* species on the basis of LD_{50}, Freq. CA, SDS Tol% and meiotic index was tried but found to be very difficult to predict and therefore suggest a mixed response.

The Chromosomal abnormalities induced from SDS treatments points to the mutagenic potential of this chemical. Therefore, observed chromosomal aberrations in the treated meiotic cells were definitely induced by SDS concentrations since such aberrations were not observed in control. This suggests that SDS could be genotoxic at the chromosomal level.

The concentration of the SDS varies in different constituents depending on its application. Moreover the concentrations of SDS used in the constituents are more and more elevated than the concentrations used in the present work. The concentrations of SDS recorded for Colgate Total, toothpaste which is used daily by the members of a house is 2400 ppm (0.24%) and for biochemical analysis in laboratories is 50,000ppm (5%) and 1,00,000ppm (10%) respectively. The elevated concentrations must have some physiological effects or alterations in the cells. Therefore, it was an attempt to record the alterations in the meiotic cells of different *Allium* species after the application of SDS at very low concentrations as compared to the 2400ppm in Colgate (toothpaste) and 5×10^4 ppm and 1×10^5 ppm in laboratories.

We may therefore,conclude that further study and screening of such hazardous chemicals should be continued at regular interval of time so that it may predict the mutagenic potential of the chemical as well as formulate strategies to prevent it from becoming a severe and harmful chemical to the cells in due course of time.

Table 1: Number of meiotic cells (Mean±S.E.), abnormal cells (Mean±S.E. and Percent), frequency chromosomal aberration (%) and SDS tolerance (%)

Concentrations (ppm)	DC	NDC	TC	Abn_{Mean}	Abn%	Freq.CA	SDS Tol%
Allium ascalonicum							
Control	**40.4±10.9**	**760.8±121.0**	**801.2±130.3**				
500ppm	**10.8±02.6**	**699.2±100.2**	**710.0±104.5**	**24.0±11.4**	**46.87**	**3.38**	**26.73**
1000 ppm	**16.2±05.7**	**660.4±151.5**	**676.6±152.9**	**36.2±11.1**	**63.95**	**5.35**	**40.09**
1500 ppm	**18.4±04.3**	**855.0±189.7**	**873.4±186.6**	**45.4±12.5**	**77.21**	**5.19**	**45.54**
Allium hookeri							
Control	**88.2±34.2**	**810.8±271.1**	**899±267.5**				
500 ppm	**34.2±21.2**	**410.6±81.1**	**456.4±78.6**	**11.6±7.2**	**09.47**	**2.54**	**38.77**
1000 ppm	**38.6±13.2**	**493.2±415.4**	**531.8±413.2**	**26.0±7.0**	**20.50**	**4.88**	**43.76**
1500 ppm	**15.2±06.0**	**451.8±74.5**	**529.2±69.4**	**62.2±12.1**	**60.15**	**11.75**	**17.23**
Allium tuberosum							
Control	**75.8±17.2**	**576.8±177.9**	**652.6±179.7**				
500 ppm	**22.6±6.9**	**382.8±73.3**	**405.4±69.5**	**20.8±5.9**	**21.13**	**5.13**	**29.81**
1000 ppm	**23.8±6.9**	**739.4±162.4**	**763.2±179.7**	**40.4±11.7**	**40.52**	**5.29**	**31.39**
1500 ppm	**38.2±6.0**	**681.8±111.2**	**720.0±116.0**	**71.6±25.1**	**62.80**	**9.94**	**50.39**
Allium porrum							
Control	**94.2±23.1**	**571.4±31.1**	**665.6±33**				
500 ppm	**24±7.2**	**624.6±82.6**	**661.2±78.1**	**12.6±3.9**	**10.6**	**1.90**	**25.4**
1000 ppm	**23±7.6**	**660.8±137.2**	**707.4±136.3**	**23.6±7.4**	**20.1**	**3.33**	**24.4**
1500 ppm	**14±5.1**	**719.2±166.2**	**780.6±177.9**	**47.4±12.5**	**43.8**	**6.07**	**14.8**
Allium chinense							
Control	**43.8±11.9**	**556.6±129.6**	**600.4±127.7**				
500 ppm	**18±2.9**	**603.8±55.5**	**621.0±191.6**	**22.2±17.7**	**35.92**	**03.57**	**41.09**
1000 ppm	**17.8±4**	**152.4±87**	**170.6±91.3**	**31.4±7.5**	**50.97**	**18.4**	**40.63**
1500 ppm	**17.2±3.9**	**177.8±96.3**	**195.0±88.7**	**42.8±12.5**	**70.16**	**21.94**	**39.26**

Dividing cells, DC; non dividing cells, NDC; total cells, TC; abnormal cells mean, Abn_{Mean}; Abnormal cells percent, Abn%; frequency of chromosomal aberrations, Freq CA; SDS salt tolerance percent, SDS Tol%.

Table 2: Lethal dose (LD_{50}) concentrations of the *Allium* species.

Allium species	Lethal Dose 50% (LD_{50}) concentration (ppm)
A. Ascalonicum	781.86
A. Hookeri	1246.80
A. Tuberosum	1194.26
A. Porrum	1712.30
A. Chinense	980.96

Table 3: Pearson correlation of dose, mean number meiotic cells and abnormalities in *A. ascalonicum.*

	Dose	DC	NDC	Abn	TC
Dose	1	-0.462	0.680	0.785	0.741
DC		1	0.153	-0.814	0.126
NDC			1	0.086	0.994**
Abn				1	0.167
TC					1

**.Correlation is significant at the 0.01 level (2-tailed)

Table 4: Pearson correlation of dose, mean number meiotic cells and abnormalities in *A. hookeri*.

	Dose	DC	NDC	Abn	TC
Dose	**1**	**-0.789**	**-0.257**	**0.996****	**-0.251**
DC		**1**	**0.226**	**-0.813**	**0.243**
NDC			**1**	**-0.180**	**0.999****
Abn				**1**	**-0.176**
TC					**1**

**.Correlation is significant at the 0.01 level (2-tailed)

Table 5: Pearson correlation of dose, mean number meiotic cells and abnormalities in *A. tuberosum*.

	Dose	DC	NDC	Abn	TC
Dose	**1**	**-0.608**	**0.668**	**0.312**	**0.571**
DC		**1**	**0.047**	**-0.450**	**0.107**
NDC			**1**	**0.449**	**0.989***
Abn				**1**	**0.515**
TC					**1**

*.Correlation is significant at the 0.05 level (2-tailed).

Table 6: Pearson correlation of dose, mean number meiotic cells and abnormalities in *A. porrum*.

	Dose	DC	NDC	Abn	TC
Dose	**1**	**-0.665**	**0.949**	**0.983***	**0.820**
DC		**1**	**-0.846**	**-0.772**	**-0.434**
NDC			**1**	**0.991****	**0.752**
Abn				**1**	**0.797**
TC					**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 7: Pearson correlation of dose, mean number meiotic cells and abnormalities in *A. chinense*.

	Dose	DC	NDC	Abn	TC
Dose	**1**	**-0.396**	**0.565**	**0.670**	**0.573**
DC		**1**	**0.413**	**-0.942**	**0.411**
NDC			**1**	**-0.171**	**1.000****
Abn				**1**	**-0.166**
TC					**1**

**.Correlation is significant at the 0.01 level (2-tailed)

Table 8: Meiotic images (Mean±S.E.) observation in different *Allium* species.

Concentrations (ppm)	Meiotic images (Mean±S.E.)
Allium ascalonicum	
Control	**40.4±17.9**
500ppm	**38.0±22.8**
1000 ppm	**36.2±16.9**
1500 ppm	**17.6±10.6**
Allium hookeri	
Control	**88.2±22.2**
500 ppm	**56.8±25.9**
1000 ppm	**26.0±14.3**
1500 ppm	**11.6±9.1**
Allium tuberosum	
Control	**75.8±29.6**
500 ppm	**74.9±53.1**
1000 ppm	**32.4±21.9**
1500 ppm	**21.0±12.5**
Allium porrum	
Control	**94.3±24.5**
500 ppm	**46.4±17.3**
1000 ppm	**24.4±10.5**
1500 ppm	**12.8±4.9**
Allium chinense	
Control	**43.8±16.9**
500 ppm	**31.6±24.4**
1000 ppm	**24.8±17.9**
1500 ppm	**13.9±8.6**

Table 9: Types of meiotic stages (Mean±S.E.) in *A. ascalonicum*

Concentration (ppm)	L	Z	P	Dipl.	Dia.	MI	AI	TI	MII	AII	TII
Control	3.8±1.4	2.8±1.7	19.8±8.2	0.4±0.4	2±1.3	-	0.4±0.4	5.2±1.4	-	-	1.8±0.8
500ppm	1±1	-	0.4±0.4	2±2	3.4±1.7	5.6±5.3	2.6±1.7	3.2±2.3	1.8±1.8	1.4±1.2	0.8±0.6
1000 ppm	0.2±0.2	-	1.2±0.9	-	7.2±4.9	5.6±3	4.2±3.2	7.8±3.7	3.6±2.1	1.2±1	3.4±1.7
1500 ppm	-	-	-	4.6±1.7	23.8±10.4	0.2±0.2	0.2±0.2	0.8±0.2	-	0.2±0.2	-

Table 10: Types of meiotic stages (Mean±S.E.) in *A. hookeri*

Concentration (ppm)	L	Z	P	Dipl.	Dia.	MI	AI	TI	MII	AII	TII	Cyt.
Control	4.2±3.2	2.4±1.4	0.4±0.2	4.4±2.3	13.2±6.6	4.6±1.6	1.2±0.7	15.4±7.9	-	-	39.8±2.1	1±0.5
500 ppm	3.4±3	-	0.8±0.4	-	2.4±1.9	0.8±0.8	-	4±2.5	-	-	0.2±0.2	-
1000 ppm	-	-	2.4±2.1	24±8	19.8±8.5	3.6±2.3	0.8±0.8	4.2±2.3	0.2±0.2		-1.8±1.1	-
1500 ppm	-	-	-	-	-	3.2±1.1	13.8±7.3	8.4±4.7	-	0.6±0.6	-	-

Table 11: Types of meiotic images (Mean±S.E.) in *A. tuberosum*

Concentration (ppm)	L	Z	P	Dipl.	Dia.	MI	AI	TI	MII	AII	TII
Control	0.6±0.6	0.2±0.2		10±7.5	28±14	0.8±0.2		28.8±1	5.2±4.2		2.2±1.7
500 ppm	0.8±0.8	0.4±0.2	0.6±0.6	2.8±1.9	11.4±7.3	8.8±4.9	22.1±20.3	13.6±4.7	4.4±4.1	6.6±5.5	3±1.8
1000 ppm	8±4.8	-	12.2±8.1	1±1	5±3.9	0.2±0.2	0.2±0.2	5.2±3	0.2±0.2	-	0.2±0.2
1500 ppm	13.2±6.7	1.4±1	4.6±3.2	-	0.6±0.4	0.2±0.2	-	0.4±0.2	-	0.4±0.2	-

Table 12: Types of meiotic stages(Mean±S.E.) in *A. porrum*

Concentration (ppm)	L	Z	P	Dipl.	Dia.	MI	AI	TI	MII	AII	TII
Control	16.0±4.1	3.2±3.11	12±13.3	18.2±19.9	19±21.4	3.4±3.3	0.6±0.8	14.6±2.1	1.2±2.1	0.6±1.3	1.1±1.7
500 ppm	3.4±0.5	0.4±0.4	2.2±0.8	1.6±0.8	2.6±1.4	1.8±1.4	0.2±0.2	-	0.2±0.2	0.2±0.2	0.2±0.2
1000 ppm	8±4.3	0.6±0.6	2±0.4	3±0.8	6.8±3.2	2±1.5	1.6±0.7	-	-	-	-
1500 ppm	17.4±4.5	6.8±3	4.4±2.2	3.4±1.2	4.4±1.5	4.6±2.4	2.8±2	-	2.2±1.5	1.4±1.2	-

Table 13: Types of meiotic stages (Mean±S.E.) in *A. chinense*

Concentration (ppm)	L	Z	P	Dipl.	Dia.	MI	AI	TI	MII	AII	TII
Control	1.8±0.8	1.6±0.5	2.6±1.1	8±3.7	16.2±4.4	0.6±0.4	1.4±0.2	5.6±2.2	0.6±0.4	1.4±0.6	2.8±1.3
500 ppm	4.2±3	1.2±0.9	2.4±1.7	1±1	1.2±0.8	15±11.3	2.6±2	-	2.2±2	2.4±2	-
1000 ppm	4.4±1.9	0.6±0.4	2.2±0.8	0.3±0.9	0.8±0.8	3±1.8	1.2±1	0.2±0.2	1.2±0.7	-	-
1500 ppm	3.6±1.9	5.4±5.4	1.4±0.6	5±4.6	4.4±2.2	1±0.4	1.6±1	-	2±1.1	0.4±0.4	-

Table 14: Different types of abnormalities in *A. ascalonicum*

Abnormalities	Concentrations		
	500ppm	1000ppm	1500ppm
St	11±6.8	19.2±6.5	20.4±8.4
Lg	1.6±1.1	4.8±2.9	2.2±0.9
Bg	1.6±0.8	0.4±0.2	1±0.7
Mmnc	5.6±0.9	7.4±4.2	10.6±5.2
Dist.	0.8±0.5	-	-
Stry.chrm	0.4±0.2	-	-
Str.shp.	0.4±0.4	-	-
Bk	0.8±0.4	1.2±0.3	1.6±0.8
2mnc	0.2±0.2	-	0.8±0.8
Rc	0.4±0.4	-	-
Uneq.dis. chrom.	1.2±1.2	-	-
Gp	-	3.2±1.3	1.2±0.5
Multipolarity	-	-	0.2±0.2
Nucm	-	-	0.4±0.4
Cytm	-	-	0.2±0.2

Table 15: Mean no.of different types of abnormalities in *A. hookeri*

Abnormalities	Concentrations		
	500ppm	1000ppm	1500ppm
St	5.8±3.3	8.2±2.7	10.6±2.9
Lg	0.2±0.2	2±0.8	0.8±0.5
Mmnc	1.6±0.6	6.6±4.4	29.4±8.6
Uneq.dis. chrm.	0.2±0.2	2.6±0.8	0.8±0.5
Nucm	2.4±2.1	-	-
Cytm	0.6±0.6	-	-
Bg	0.2±0.2	2.4±0.7	1.6±0.9
2mnc	0.2±0.2	-	-
Str.shp.	0.2±0.2	0.2±0.2	0.2±0.2
Frg	0.2±0.2	2±0.8	0.8±0.3
Dist.	-	1.2±0.3	0.2±0.2
Split chrm.	-	0.2±0.2	-
Mult.	-	0.6±0.4	0.4±0.4
Rc	-	-	2.6±1.5
Gp	-	-	3.8±1.1
2mnc	-	-	5.4±2
3mnc	-	-	0.2±0.2
Nucm	-	-	2.4±1.7
Ctym	-	-	3±2.3

Table 16: Mean no.of different types of abnormalities in *A. tuberosum*

Abnormalities	Concentrations		
	500ppm	1000ppm	1500ppm
St	13.4±3.6	39.6±11.2	10.8±1.5
Mnc	-	18.2±14.3	-
Frg	-	1.2±0.8	-
Lg	1.4±1.4	0.6±0.4	0.4±0.4
Dist.	-	0.2±0.2	-
Mult.	-	0.8±0.8	-
Stry.chrm.	-	0.6±0.6	-
Gp	0.8±0.8	0.6±0.4	0.2±0.2
Uneq.dis. chrm.	3.4±2.1	2.4±1.9	-
Bg	0.6±0.2	0.8±0.3	0.2±0.2
Rc	1.2±1.2	1.4±1.4	19±11
Transl.zone	-	-	-
Nucm	-	4.2±3	0.8±0.8
Mac.	-	1±1	-

Table 17: Different types of abnormalities (Mean±S.E.) in *A. porrum*

Abnormalities	Concentrations		
	500ppm	1000ppm	1500ppm
St	11.4±3.3	20.8±7.6	37.2±12.3
Frg	0.4±0.2	1.2±0.3	2.2±0.8
Rc	0.4±0.4	-	1.4±0.5
Mnc	0.2±0.2	-	-
Uneq.dis.chrm.	0.2±0.2	0.8±0.5	2.2±1
Gp	-	0.8±0.3	2.4±0.8
Bg	-	-	2±0.9

Table 18: Mean no.of different types of abnormalities in *A. chinense*

Abnormalities	Concentrations		
	500ppm	1000ppm	1500ppm
St	16±9.4	14.2±5.2	13.4±3.4
Mnc	1±0.6	0.2±0.2	9.6±9.6
Frg	3.2±2.1	0.2±0.2	0.2 ±0.2
Lg	0.4±0.2	-	-
Dist.	0.2±0.2	0.2±0.2	-
Mult.	0.4±0.2	-	0.2±0.2
Stry.chrm.	0.4±0.4	-	-
Loop form	0.8±0.5	-	-
Loose end mat.	1.2±1.2	-	-
Twst.chrm	1±0.6	-	-
Inter.chrm	1.2±0.5	0.4±0.4	-
Trans.zone	1±0.7	-	-
Gp	1±0.7	0.4±0.2	
Uneq.dis. chrom.	0.6±0.6	-	0.8±0.2
Uni	0.6±0.6	-	
Biv	0.4±0.4	-	-
Bk	0.8±0.8	-	-
Bg	0.6±0.6	0.2±0.2	0.6±0.4
Rc	0.4±0.4	0.4±0.4	-
Transl.zone	0.2±0.2	-	-
Nucm	-	5.4±2.6	7±1.7
Cytm	-	0.2±0.2	1.6±0.6
Uneq.dis.chrm	-	0.4±0.4	-

Table 19: Pearson Correlation of types of abnormalities in *A. ascalonicum* at 500ppm

	St	Lg	Bg	Mnc	Dist.	Stry.chrm.	Star shape	Frg	2mnc	Rc	Uneq.dis. chrm.
St	**1**	**0.954***	**0.396**	**0.189**	**0.954***	**-0.388**	**0.988****	**0.657**	**-0.366**	**0.988****	**0.988****
Lg		**1**	**0.380**	**-0.037**	**1.000****	**-0.210**	**0.943***	**0.840**	**-0.343**	**0.943***	**0.943***
Bg			**1**	**-0.451**	**0.380**	**0.452**	**0.431**	**0.201**	**-0.492**	**0.431**	**0.431**
Mnc				**1**	**-0.037**	**-0.704**	**0.108**	**-0.264**	**-0.162**	**0.108**	**0.108**
Dist.					**1**	**-0.210**	**0.943***	**0.840**	**-0.343**	**0.943***	**0.943***
Stry.chrm.						**1**	**-0.408**	**0.167**	**-0.408**	**-0.408**	**-0.408**
Star shape							**1**	**0.612**	**-0.250**	**1.000****	**1.000****
Frg.								**1**	**-0.408**	**0.612**	**0.612**
2mnc									**1**	**-0.250**	**-0.250**
Rc										**1**	**1.000****
Uneq.dis.chrm.											**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 20: Pearson Correlation of types of abnormalities in *A. ascalonicum* at 1000ppm

	St	Lg	Bg	Mnc	Gp	Frg
St	**1**	**0.964****	**0.733**	**0.125**	**-0.716**	**0.622**
Lg		**1**	**0.587**	**-0.091**	**-0.722**	**0.721**
Bg			**1**	**0.732**	**-0.764**	**0.090**
Mnc				**1**	**-0.233**	**-0.343**
Gp					**1**	**-0.118**
Frg						**1**

**.Correlation is significant at the 0.01 level (2-tailed)

Table 21: Pearson Correlation of types of abnormalities in *A. ascalonicum* at 1500ppm

	St	Lg	Bg	Mnc	Mult.	Nucm	Cytm	Gp	Frg	2mnc
St	**1**	**0.010**	**0.442**	**0.210**	**0.412**	**0.218**	**0.519**	**0.696**	**0.696**	**-0.395**
Lg		**1**	**0.599**	**0.346**	**0.152**	**-0.283**	**0.722**	**-0.567**	**-0.567**	**0.206**
Bg			**1**	**-0.025**	**0.874**	**0.554**	**0.968****	**-0.323**	**-0.323**	**-0.323**
Mnc				**1**	**-0.289**	**-0.489**	**0.126**	**0.126**	**0.126**	**0.805**
Mult.					**1**	**0.887***	**0.739**	**-0.185**	**-0.185**	**-0.492**
Nucm						**1**	**0.343**	**-0.086**	**-0.086**	**-0.514**
Cytm							**1**	**-0.250**	**-0.250**	**-0.250**
Gp								**1**	**1.000****	**-0.250**
Frg									**1**	**-.250**
2mnc										**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed

Table 22: Pearson Correlation of types of abnormalities in *A. hookeri* at 500ppm

	St	Lg	Mnc	Uneq.dis.chrm.	Nucm	Cytm	Bg	2mnc	Star shape	Frg
St	**1**	**0.971****	**0.447**	**0.971****	**0.985****	**0.971****	**-0.059**	**-0.059**	**0.971****	**0.971****
Lg		**1**	**0.516**	**1.000****	**0.996****	**1.000****	**-0.250**	**-0.250**	**1.000****	**1.000****
Mnc			**1**	**0.516**	**0.540**	**0.516**	**0.147**	**0.147**	**0.516**	**0.516**
Uneq.dis. chrm.				**1**	**0.996****	**1.000****	**-0.250**	**-0.250**	**1.000****	**1.000****
Nucm					**1**	**0.996****	**-0.162**	**-0.162**	**0.996****	**0.996****
Cytm						**1**	**-0.250**	**-0.250**	**1.000****	**1.000****
Bg							**1**	**1.000****	**-0.250**	**-0.250**
2mnc								**1**	**-0.250**	**-0.250**
Str. Shp.									**1**	**1.000****
Frg										**1**

**.Correlation is significant at the 0.01 level (2-tailed)

Table 23*:* Pearson Correlation of types of abnormalities in *A. hookeri* at 1000ppm

	St	Lg	Bg	Mnc	Dist.	Mult.	Uneq.dis. chrm.	Split chrm.	Str. Shp.	Frg
St	**1**	**-0.218**	**0.039**	**-0.209**	**-0.545**	**0.109**	**0.071**	**0.164**	**0.164**	**-0.131**
Lg		**1**	**0.080**	**0.519**	**0.799**	**-0.896***	**0.137**	**-0.598**	**-0.598**	**0.643**
Bg			**1**	**0.786**	**0.464**	**-0.367**	**0.904***	**-0.468**	**-0.468**	**0.799**
Mnc				**1**	**0.696**	**-0.551**	**0.897***	**-0.367**	**-0.367**	**0.958***
Dist.					**1**	**-0.869**	**0.368**	**-0.802**	**-0.802**	**0.799**
Mult.						**1**	**-0.258**	**0.875**	**0.875**	**-0.747**
Uneq.dis.chrm							**1**	**-0.172**	**-0.172**	**0.823**
Split chrm.								**1**	**1.000****	**-0.598**
Str.Shp.									**1**	**-0.598**
Frg										**1**

*.Correlation is significant at the 0.05 level (2-tailed

**.Correlation is significant at the 0.01 level (2-tailed)

Table 24: Pearson Correlation of types of abnormalities in *A. hookeri* at 1500ppm

	St	Bg	Lg	Mnc	2mnc	Dist.	Mult.	Rc	Gp	Star shape	Frg	Nucm	Cytm	3mnc	Uneq.dis. chrm.
St	**1**	**-0.232**	**-0.328**	**0.367**	**0.618**	**-0.470**	**-0.470**	**0.276**	**0.067**	**0.706**	**-0.198**	**-0.242**	**-0.304**	**-0.470**	**0.680**
Bg		**1**	**0.980****	**-0.753**	**-0.831**	**-0.431**	**-0.431**	**-0.414**	**-0.065**	**-0.162**	**0.807**	**-0.499**	**-0.487**	**-0.431**	**0.148**
Lg			**1**	**-0.717**	**-0.805**	**-0.343**	**-0.343**	**-0.357**	**-0.015**	**-0.343**	**0.871**	**-0.471**	**-0.443**	**-0.343**	**-0.029**
Mnc				**1**	**0.850**	**-0.156**	**-0.156**	**0.907***	**0.684**	**-0.040**	**-0.286**	**-0.174**	**-0.171**	**-0.156**	**-0.292**
2mnc					**1**	**0.072**	**0.072**	**0.636**	**0.277**	**0.192**	**-0.486**	**0.140**	**0.124**	**0.072**	**-0.025**
Dist.						**1**	**1.000****	**-0.423**	**-.605**	**-0.250**	**-0.535**	**0.943***	**0.968****	**1.000****	**-0.343**
Mult.							**1**	**-0.423**	**-0.605**	**-0.250**	**-0.535**	**0.943***	**0.968****	**1.000****	**-0.343**
Rc								**1**	**0.917***	**-0.260**	**0.139**	**-0.525**	**-0.504**	**-0.423**	**-0.413**
Gp									**1**	**-0.389**	**0.439**	**-0.756**	**-0.725**	**-0.605**	**-0.459**
Star shape										**1**	**-0.535**	**0.086**	**0.000**	**-0.250**	**0.943***
Frg											**1**	**-0.733**	**-0.690**	**-0.535**	**-0.275**
Nucm												**1**	**0.996****	**0.943***	**-0.029**
Cytm													**1**	**0.968****	**-0.111**
3mnc														**1**	**-0.343**
Uneq.dis.chrm.															**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 25: Pearson Correlation of types of abnormalities in *A. tuberosum* at 500ppm

	St	uneq.dis. of chrm.	Lg	Bg	Gp	Rc
St	**1**	**0.917***	**0.794**	**0.781**	**0.643**	**0.626**
uneq.dis. of chrm.		**1**	**0.866***	**0.746**	**0.700**	**0.313**
Lg			**1**	**0.791**	**0.250**	**0.250**
Bg				**1**	**0.316**	**0.316**
Gp					**1**	**0.250**
Rc						**1**

***.** Correlation is significant at the 0.05 level (2-tailed).

Table 26: Pearson Correlation of types of abnormalities in *A. tuberosum* at 1000ppm

	St	Mnc	Mac	Nucm	Frg	Lg	Bg	Rc	Uneq. Dis.chm.	Mult.	Stry. chrm	Dist.	Gp
St	**1**	**0.319**	**0.343**	**0.166**	**0.303**	**0.303**	**0.055**	**0.722**	**0.762**	**0.722**	**0.722**	**0.722**	**0.080**
Mnc		**1**	**0.991****	**0.985****	**-0.424**	**-0.424**	**-0.623**	**-0.213**	**-0.152**	**-0.213**	**-0.213**	**-0.213**	**-0.424**
Mac			**1**	**0.970****	**-0.375**	**-0.375**	**-0.535**	**-0.250**	**-0.183**	**-0.250**	**-0.250**	**-0.250**	**-0.375**
Nucm				**1**	**-0.436**	**-0.436**	**-0.695**	**-0.345**	**-0.296**	**-0.345**	**-0.345**	**-0.345**	**-0.518**
Frg					**1**	**1.000****	**0.200**	**0.250**	**0.183**	**0.250**	**0.250**	**0.250**	**-0.250**
Lg						**1**	**0.200**	**0.250**	**0.183**	**0.250**	**0.250**	**0.250**	**-0.250**
Bg							**1**	**0.134**	**0.168**	**0.134**	**0.134**	**0.134**	**0.869**
Rc								**1**	**0.993****	**1.000****	**1.000****	**1.000****	**0.250**
Uneq.dis. Chrm									**1**	**0.993****	**0.993****	**0.993****	**0.314**
Mult.										**1**	**1.000****	**1.000****	**0.250**
Stry.chrm.											**1**	**1.000****	**0.250**
Dist.												**1**	**0.250**
Gp													**1**

**. Correlation is significant at the 0.01 level (2-tailed).

Table 27: Pearson Correlation of types of abnormalities in *A. tuberosum* at 1500ppm

	St	Lg	Gp	Rc	Nucm	Bg
St	**1**	**0.502**	**0.502**	**0.076**	**-0.125**	**-0.439**
Lg		**1**	**1.000****	**-0.406**	**-0.250**	**-0.250**
Gp			**1**	**-0.406**	**-0.250**	**-0.250**
Rc				**1**	**0.925***	**-0.256**
Nuclm					**1**	**-0.250**
Bg						**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 28: Pearson Correlation of types of abnormalities in 500ppm of *A. porrum*

	St	Frg	Rc	Mnc	Uneq.dis. chrm.
St	**1**	**0.856**	**0.192**	**0.192**	**0.857**
Frg		**1**	**0.612**	**0.612**	**0.612**
Rc			**1**	**1.000****	**-0.250**
Mnc				**1**	**-0.250**
Uneq.dis. chrm					**1**

**.Correlation is significant at the 0.01 level (2-tailed).

Table 29: Pearson Correlation of types of abnormalities in *A. porrum* at 1000ppm

	St	Frg	Gp	Uneq.dis.chrm.
St	**1**	**0.250**	**-0.197**	**-0.239**
Frg		**1**	**-0.286**	**-0.871**
Gp			**1**	**-0.046**
Uneq.dis.chrm.				**1**

Table 30: Pearson Correlation of types of abnormalities in *A. porrum* at 1500ppm

	St	Frg	Gp	Uneq.dis. chrm.	Rc	Bg
St	**1**	**0.751**	**-0.072**	**-0.135**	**0.622**	**-0.702**
Frg		**1**	**0.277**	**-0.257**	**0.932***	**-0.132**
Gp			**1**	**-0.748**	**0.266**	**0.454**
Uneq.dis. chrm.				**1**	**-0.423**	**-0.362**
Rc					**1**	**0.103**
Bg						**1**

*. Correlation is significant at the 0.05 level (2-tailed).

Table 31: Pearson Correlation of types of abnormalities in *A. chinense* at 500ppm

	St	Mnc	Frg	Lg	Dist.	Mult	Stry. chm.	Loop form.	Looe end mat.	Twst. chrm	Inter. chrm	Trans Chrm	Gp	Uneq. dis. chrm.	Uni	Biv	Bk	Bg	Rc	trans. chrm
St	1	0.748	0.603	0.890*	0.904*	0.890*	0.904*	0.501	0.904*	0.748	0.675	0.425	0.981**	0.904*	0.186	0.186	0.186	0.186	0.186	0.186
Mnc		1	0.980**	0.968**	0.395	0.968**	0.395	0.949*	0.395	1.000**	0.949*	0.919*	0.612	0.395	0.791	0.791	0.791	0.791	0.791	0.791
Frg			1	0.900*	0.207	0.900*	0.207	0.993**	0.207	0.980**	0.937*	0.978**	0.445	0.207	0.896*	0.896*	0.896*	0.896*	0.896*	0.896*
Lg				1	0.612	1.000**	0.612	0.840	0.612	0.968**	0.910*	0.791	0.791	0.612	0.612	0.612	0.612	0.612	0.612	0.612
Dist.					1	0.612	1.000**	0.086	1.000**	0.395	0.343	0.000	0.968**	1.000**	-0.250	-0.250	-0.250	-0.250	-0.250	-0.250
Mult.						1	0.612	0.840	0.612	0.968**	0.910*	0.791	0.791	0.612	0.612	0.612	0.612	0.612	0.612	0.612
stry. chrm							1	0.086	1.000**	0.395	0.343	0.000	0.968**	1.000**	-0.250	-0.250	-0.250	-0.250	-0.250	-0.250
Loop form.								1	0.086	0.949*	0.912*	0.996**	0.332	0.086	0.943*	0.943*	0.943*	0.943*	0.943*	0.943*
Loose end mat.									1	0.395	0.343	0.000	0.968**	1.000**	-0.250	-0.250	-0.250	-0.250	-0.250	-0.250
Twst. chrm.										1	0.949*	0.919*	0.612	0.395	0.791	0.791	0.791	0.791	0.791	0.791
Inter. chrm.											1	0.886*	0.554	0.343	0.772	0.772	0.772	0.772	0.772	0.772
Trans. chrm.												1	0.250	0.000	0.968**	0.968**	0.968**	0.968**	0.968**	0.968**

Gp													**1**	**0.968****	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Uneq. dis. chrm.														**1**	**-0.250**	**-0.250**	**-0.250**	**-0.250**	**-0.250**	**-0.250**
Uni															**1**	**1.000****	**1.000****	**1.000****	**1.000****	**1.000****
Biv																**1**	**1.000****	**1.000****	**1.000****	**1.000****
Bk																	**1**	**1.000****	**1.000****	**1.000****
Bg																		**1**	**1.000****	**1.000****
Rc																			**1**	**1.000****
trans. chrm.																				**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 32: Pearson Correlation of abnormalities in *A. chinense* at 1000ppm

	St	Mnc	Frg	Dist.	Inter.chrm.	Gp	Uneq.dis. chrm.	Rc	Bg	Nucm	Cytm
St	**1**	**0.652**	**0.557**	**0.557**	**0.652**	**-0.748**	**0.652**	**-0.529**	**0.652**	**0.525**	**0.557**
Mnc		**1**	**-0.250**	**-0.250**	**1.000****	**-0.408**	**1.000****	**-0.250**	**1.000****	**-0.133**	**-0.250**
Frg			**1**	**1.000****	**-0.250**	**-0.408**	**-0.250**	**-0.250**	**-0.250**	**0.720**	**1.000****
Dist.				**1**	**-0.250**	**-0.408**	**-0.250**	**-0.250**	**-0.250**	**0.720**	**1.000****
Inter.chrm					**1**	**-0.408**	**1.000****	**-0.250**	**1.000****	**-0.133**	**-0.250**
Gp						**1**	**-0.408**	**0.612**	**-0.408**	**-0.836**	**-0.408**
Uneq.dis.chrm							**1**	**-0.250**	**1.000****	**-0.133**	**-0.250**
Rc								**1**	**-0.250**	**-0.512**	**-0.250**
Bg									**1**	**-0.133**	**-0.250**
Nucm										**1**	**0.720**
Cytm											**1**

**.Correlation is significant at the 0.01 level (2-tailed)

Table 33: Pearson Correlation of types of abnormalities in *A. chinense* at 1500ppm

	St	Mnc	Frg	Bg	Nucm	Cytm	Uneq.dis. chrm.	Mult.
St	**1**	**0.410**	**0.117**	**0.029**	**-0.254**	**0.147**	**0.249**	**0.410**
Mnc		**1**	**-0.250**	**-0.375**	**0.140**	**0.147**	**0.250**	**1.000****
Frg			**1**	**-0.375**	**-0.978****	**-0.221**	**0.250**	**-0.250**
Bg				**1**	**0.489**	**0.774**	**-0.875**	**-0.375**
Nucm					**1**	**0.330**	**-0.419**	**0.140**
Cytm						**1**	**-0.885***	**0.147**
Uneq.dis.chrm.							**1**	**0.250**
Mult.								**1**

*.Correlation is significant at the 0.05 level (2-tailed)

**.Correlation is significant at the 0.01 level (2-tailed)

Table 34: Physiological types of abnormalities in *Allium* species

Concentrations (ppm)	Laggard	Stickiness	Strayed Chromosome	Abnormal Anaphase	Abnormal Metaphase
Allium ascalonicum					
Control					
500ppm	**1.60±1.16**	**11.0±6.83**	**0.40±0.24**	**0.20±0.20**	**1.80±1.80**
1000 ppm	**4.80±2.92**	**19.20±6.56**	**0±0**	**0±0**	**0±0**
1500 ppm	**2.20±0.96**	**20.40±8.47**	**0±0**	**0±0**	**0.8±0.48**
Allium hookeri					
Control					
500 ppm	**0.2±0.2**	**5.8±3.39**	**0.2±0.2**	**0±0**	**0.4±0.24**
1000 ppm	**2.0±2.0**	**8.20±2.74**	**2.0±0.83**	**3.4±0.92**	**0±0**
1500 ppm	**0.80±0.58**	**10.60±2.97**	**0.8±0.58**	**0.4±0.4**	**0.40±0.24**
Allium tuberosum					
Control					
500 ppm	**1.40±1.40**	**13.40±3.69**	**0±0**	**0.20±0.20**	**0±0**
1000 ppm	**0.60±0.40**	**39.60±11.21**	**0.60±0.60**	**1.0±1.0**	**0±0**
1500 ppm	**0.40±0.40**	**10.80±1.59**	**0±0**	**0±0**	**0±0**
Allium porrum					
Control					
500 ppm	**0±0**	**11.40±3.35**	**0±0**	**0±0**	**0.4±0.40**
1000 ppm	**0±0**	**20.80±7.60**	**0±0**	**1.60±0.509**	**0.4±0.24**
1500 ppm	**0±0**	**37.20±12.39**	**0±0**	**1.80±1.356**	**5.0±0.83**
Allium chinense					
Control					
500 ppm	**0.40±0.24**	**16.0±9.40**	**0.40±0.40**	**0.40±0.40**	**7.80±5.20**
1000 ppm	**0±0**	**14.20±5.29**	**0±0**	**0.40±0.40**	**0.80±0.80**
1500 ppm	**0±0**	**13.40±3.41**	**0±0**	**0.2±0.2**	**0.80±0.37**

Table 35: Clastogenic types of abnormalities in *Allium* species

Concentrations (ppm)	Chromosome Breaks	Ring Chromosomes	Micronuclei
Allium ascalonicum			
Control			
500 ppm	**0.8±0.48**	**0.40±0.40**	**5.6±0.92**
1000 ppm	**1.2±0.37**	**0±0**	**7.4±4.23**
1500 ppm	**1.6±0.81**	**0±0**	**12.4±5.15**
Allium hookeri			
Control			
500 ppm	**0.2±0.2**	**0±0**	**1.6±0.50**
1000 ppm	**2±0.83**	**0±0**	**6.6±4.48**
1500 ppm	**0.8±0.37**	**2.6±1.53**	**35±1.09**
Allium tuberosum			
Control			
500 ppm	**0±0**	**1.20±1.20**	**0±0**
1000 ppm	**1.2±0.80**	**1.40±1.40**	**18.20±14.32**
1500 ppm	**0±0**	**19.0±11.07**	**0±0**
Allium porrum			
Control			
500 ppm	**0±0**	**0.40±0.40**	**0.20±0.20**
1000 ppm	**0±0**	**0±0**	**0±0**
1500 ppm	**0±0**	**1.40±0.50**	**0±0**
Allium chinense			
Control			
500 ppm	**0.80±0.80**	**0.40±0.40**	**1.0±0.63**
1000 ppm	**0±0**	**0.40±0.40**	**0.20±0.20**
1500 ppm	**0±0**	**0±0**	**9.60±9.60**

Table 36: Meiotic index at different SDS concentrations in different *Allium* species

Concentrations (ppm)	Meiotic index (%)
Allium ascalonicum	
Control	**5.04**
500ppm	**1.52**
1000ppm	**2.39**
1500ppm	**2.10**
Allium hookeri	
Control	**9.81**
500ppm	**7.49**
1000ppm	**7.25**
1500ppm	**2.87**
Allium tuberosum	
Control	**11.61**
500ppm	**5.57**
1000ppm	**3.11**
1500ppm	**5.30**
Allium porrum	
Control	**14.15**
500ppm	**3.62**
1000ppm	**3.25**
1500ppm	**1.79**
Allium chinense	
Control	**7.29**
500ppm	**2.89**
1000ppm	**10.43**
1500ppm	**8.82**

Meiotic abnormalities in *A.ascalonicum*

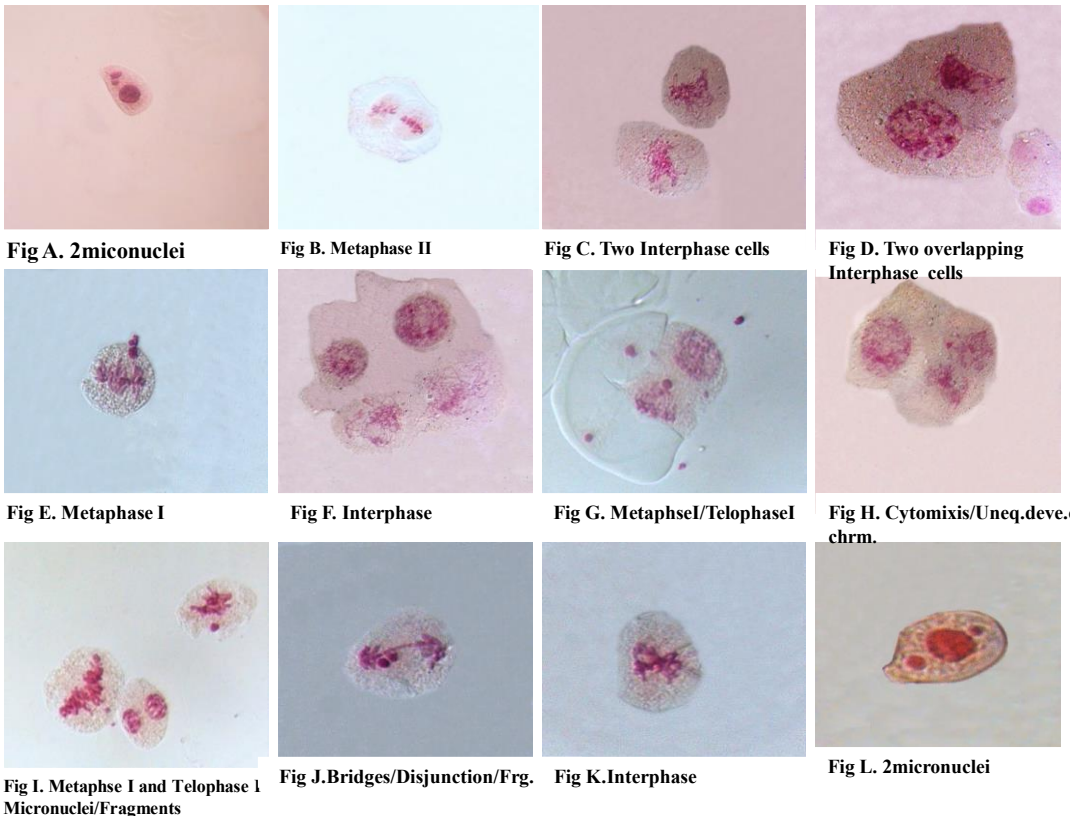


Figure Plate 1: Various types of abnormalities in *A. ascalonicum*

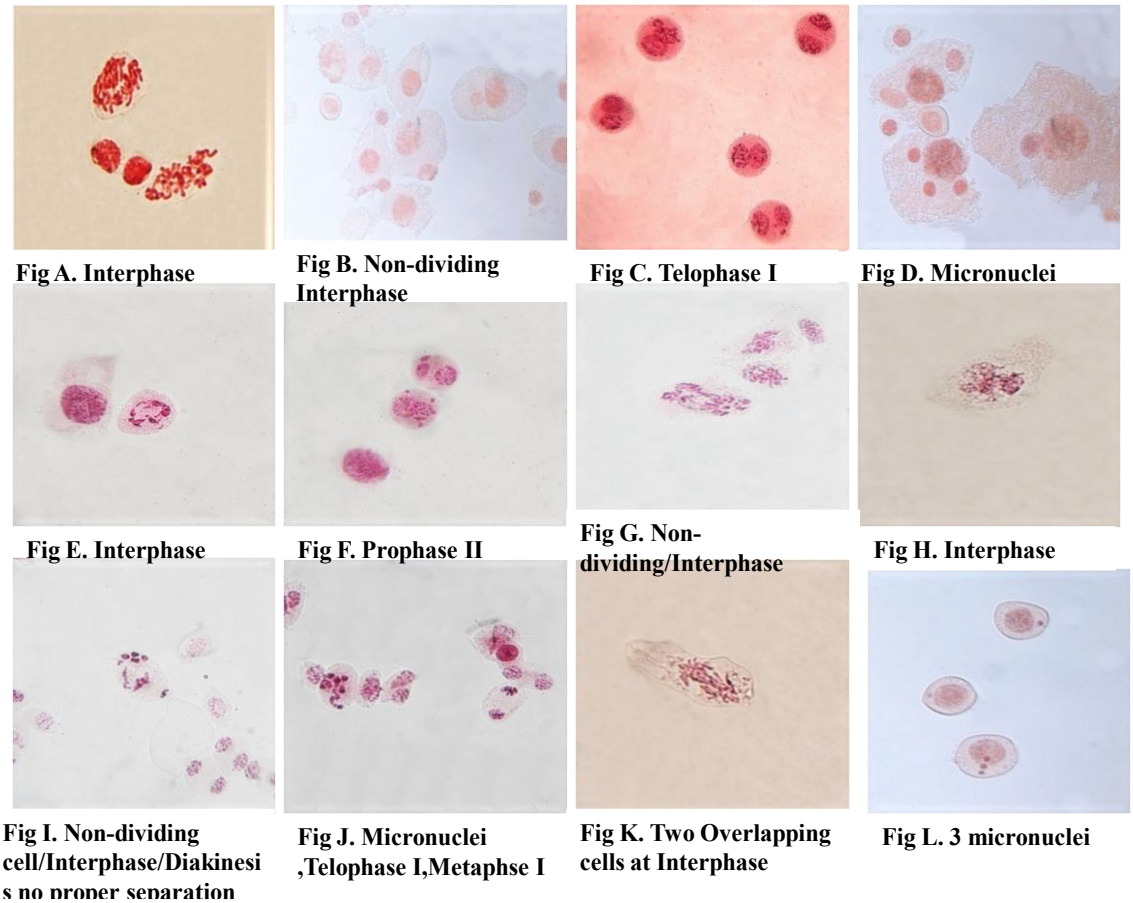


Figure Plate 2: Various types of abnormalities in *A. hookeri*

Meiotic abnormalities in *A.tuberosum*

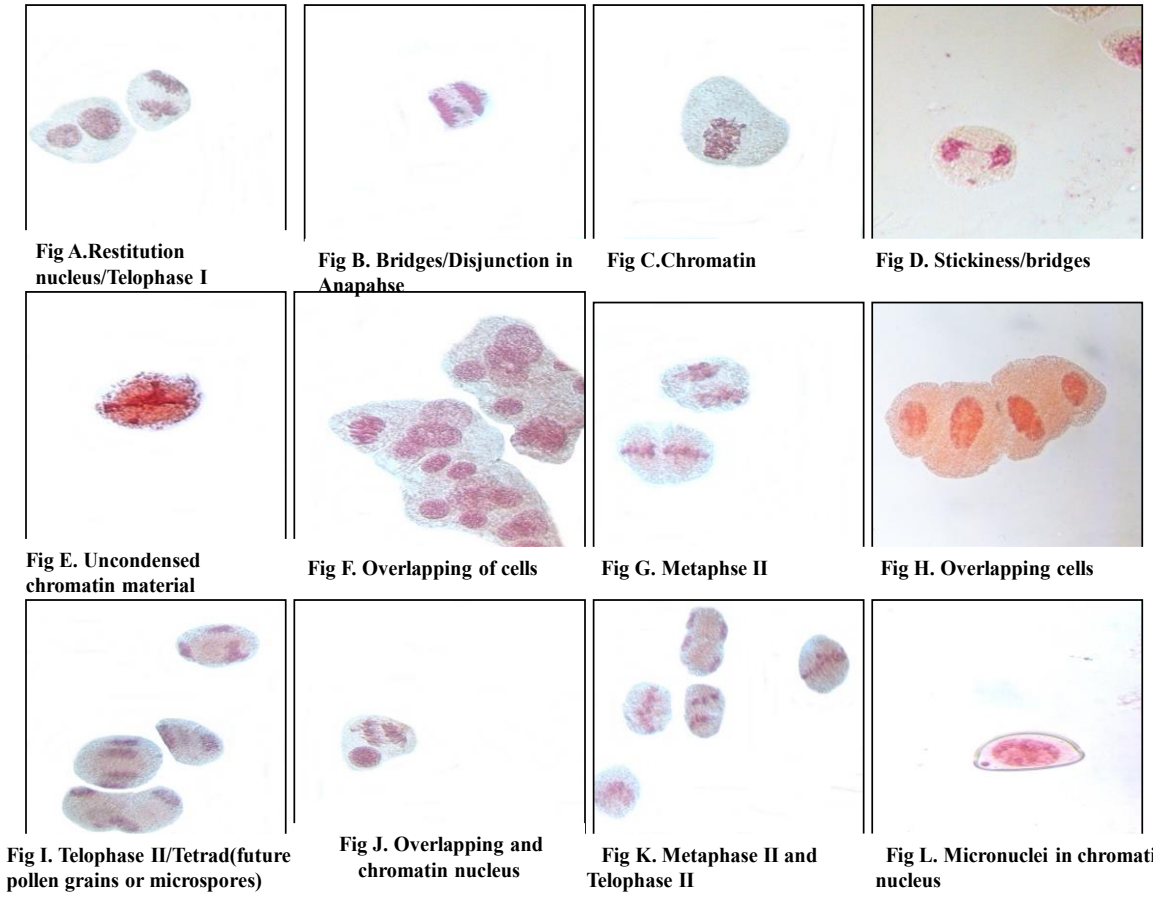


Figure Plate 3: Various types of abnormalities in *A. tuberosum*

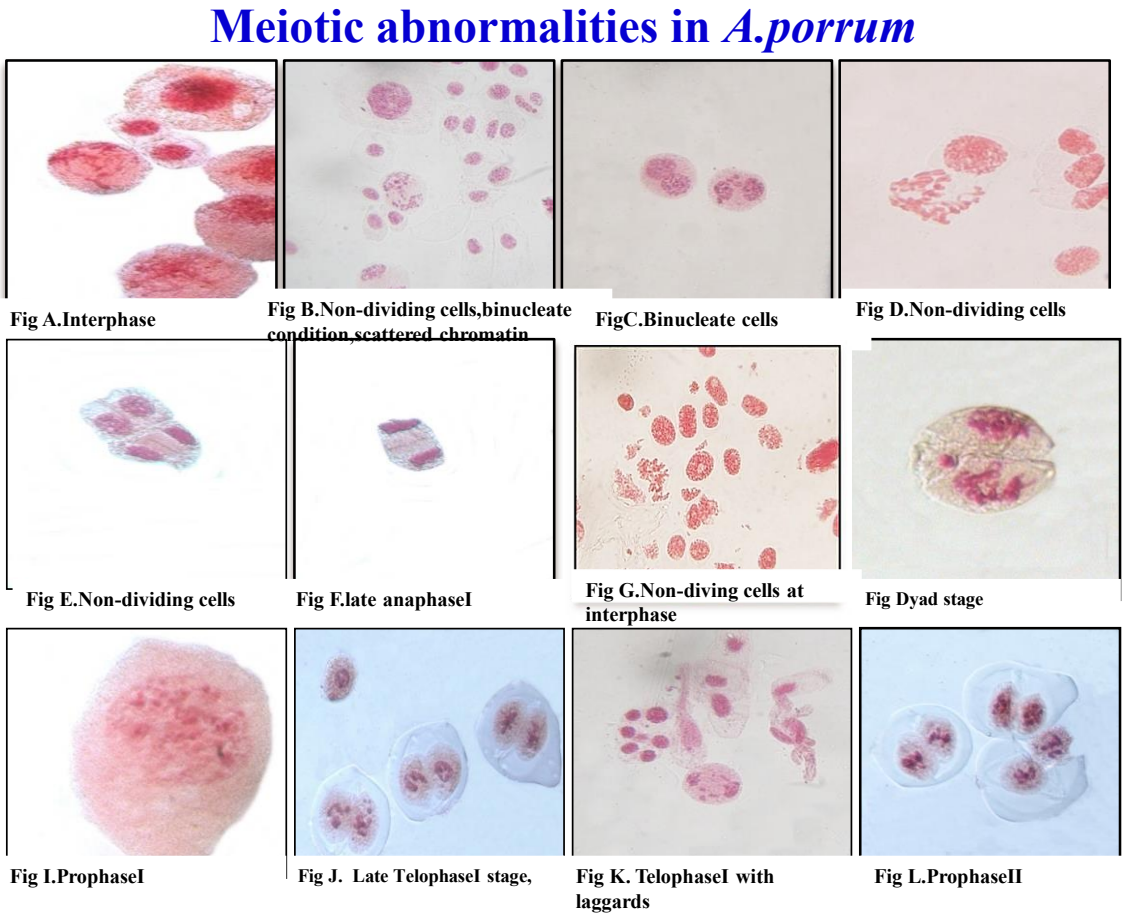


Figure Plate 4: Various types of abnormalities in *A. porrum*

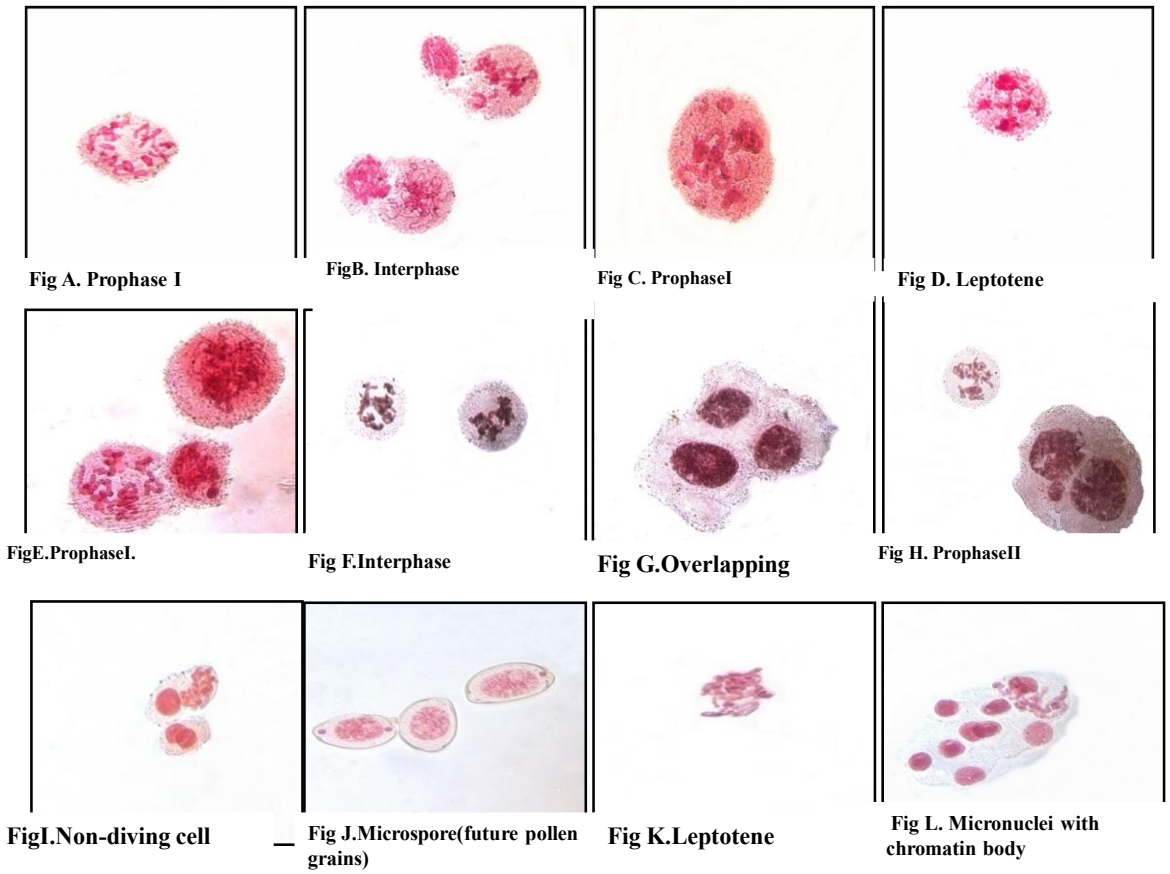
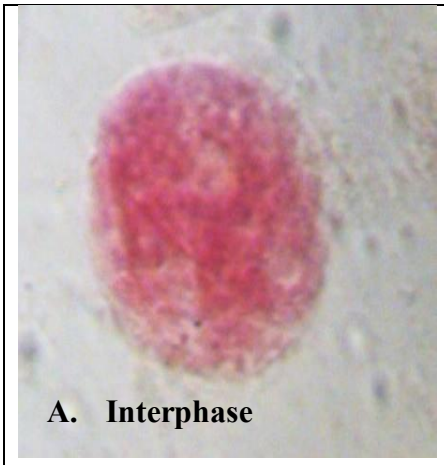
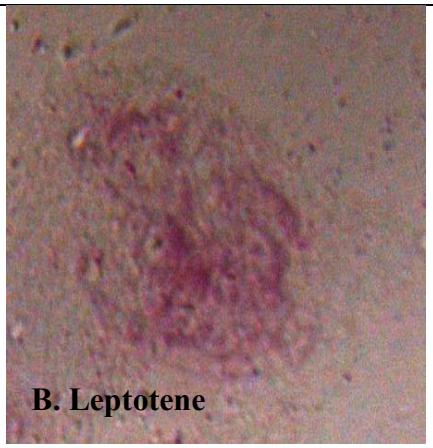
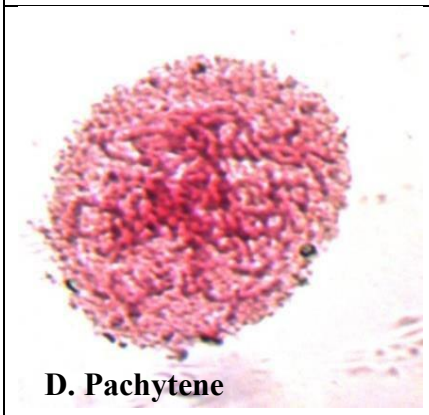


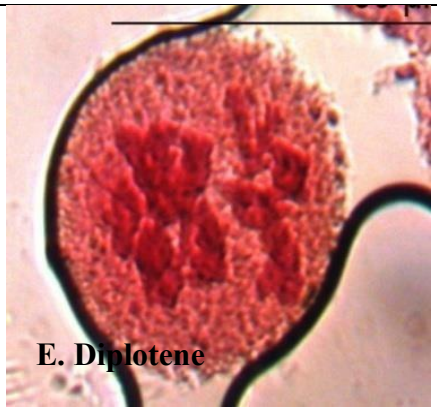
Figure Plate 5: Various types of abnormalities in *A. chinense*

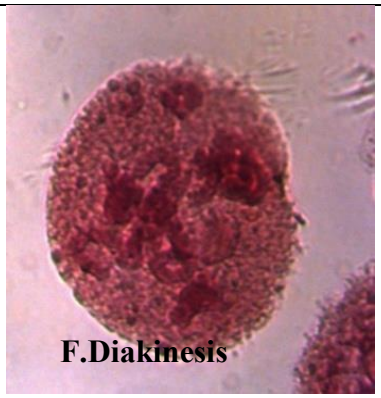

A. Interphase

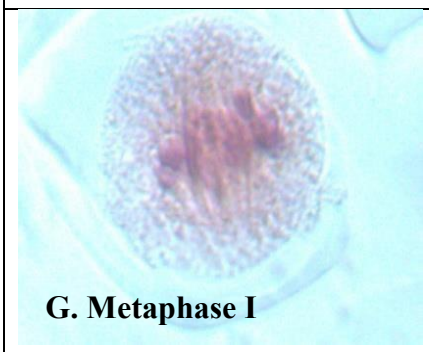

B. Leptotene

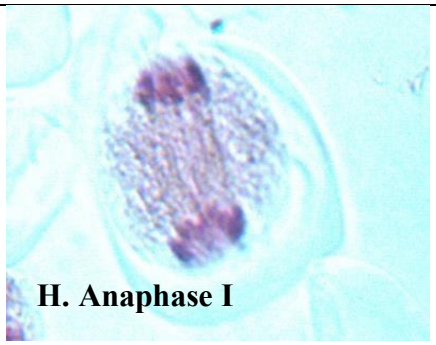

C. Zygotene


D. Pachytene


E. Diplotene


F.Diakinesis


G. Metaphase I


H. Anaphase I


I.Telophase I

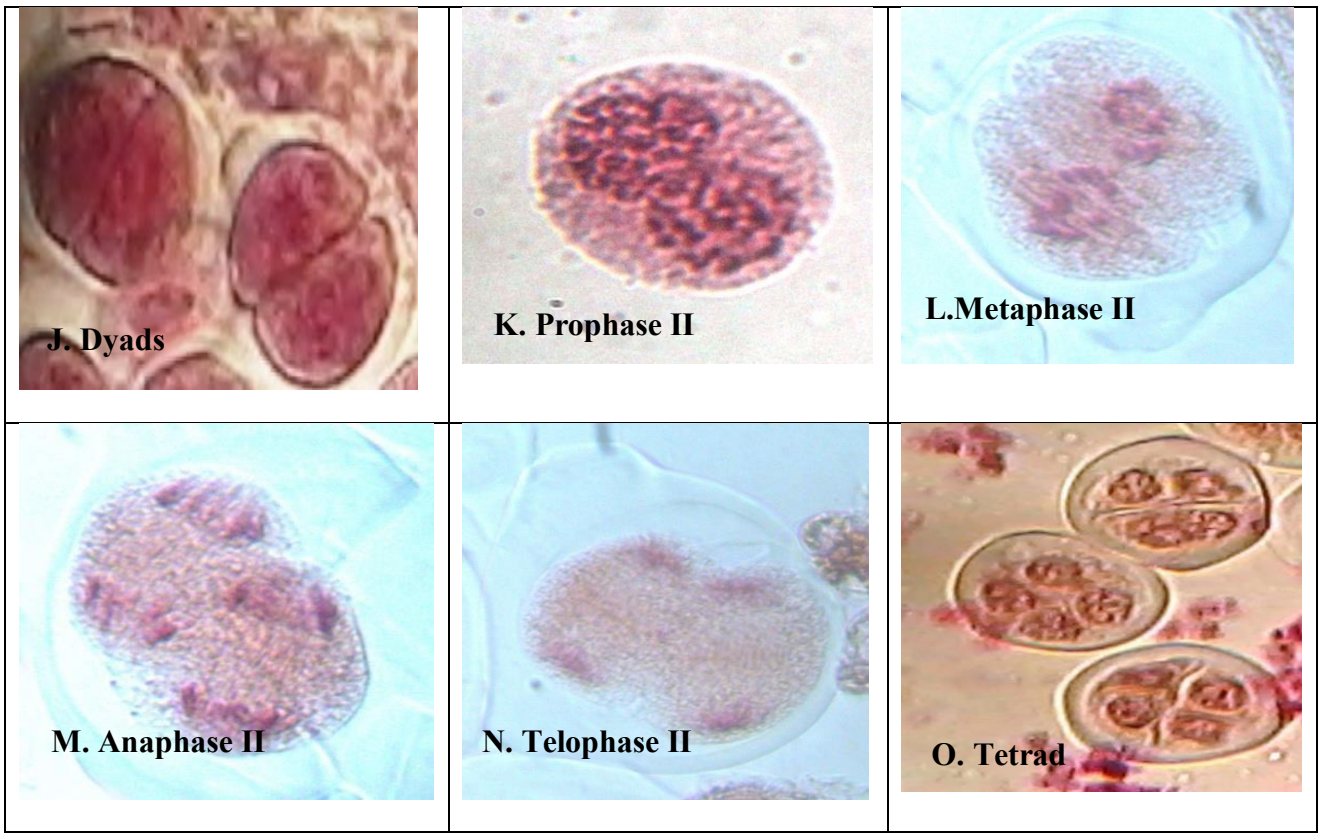


Figure Plate 6: Various stages of meiosis of *Allium*. A-J Meiosis I; K-O Meiosis II.

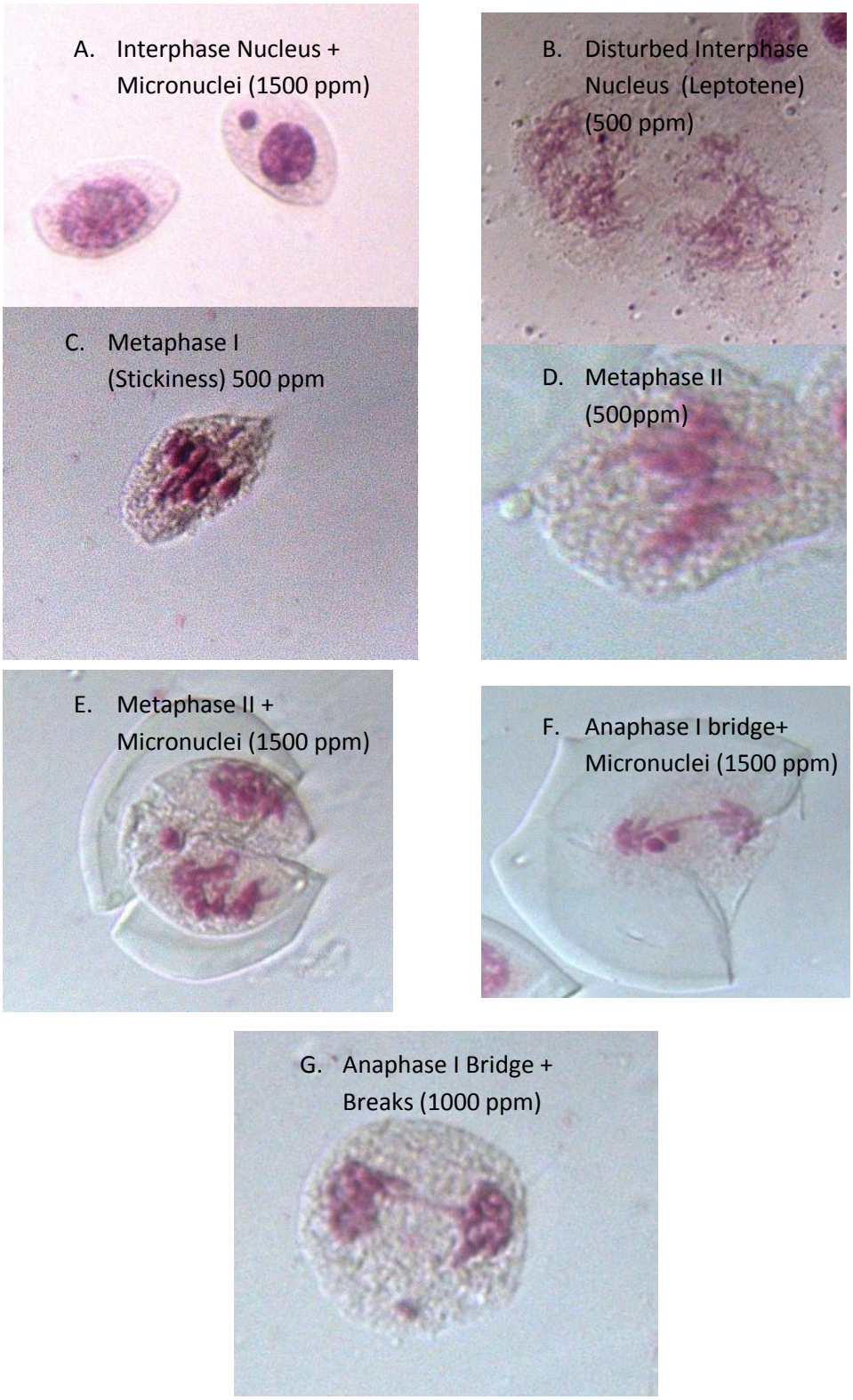
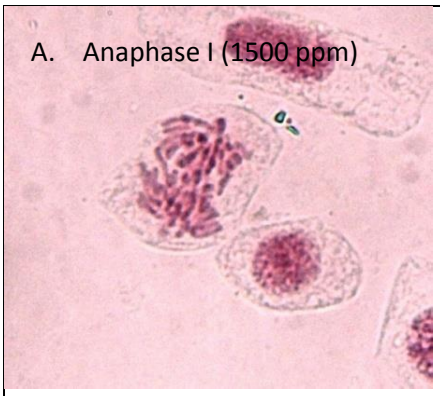
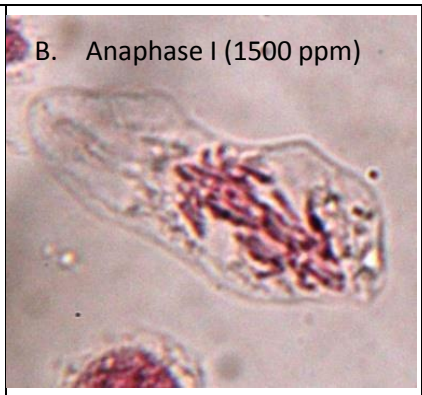
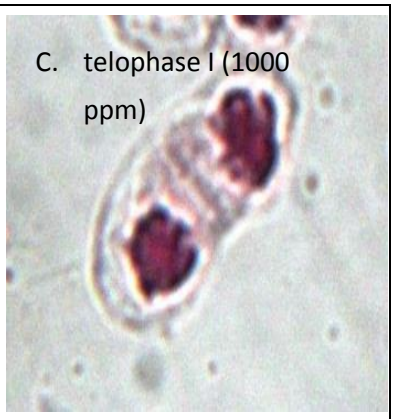
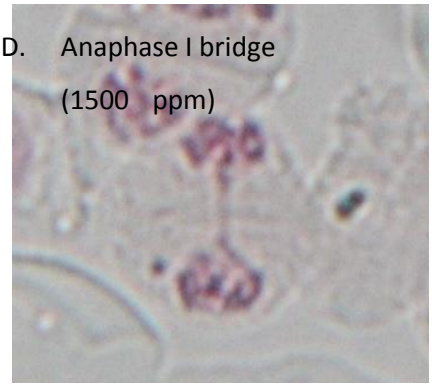
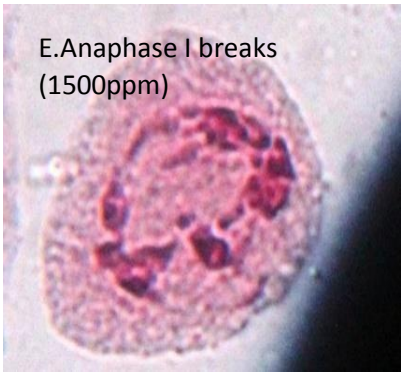
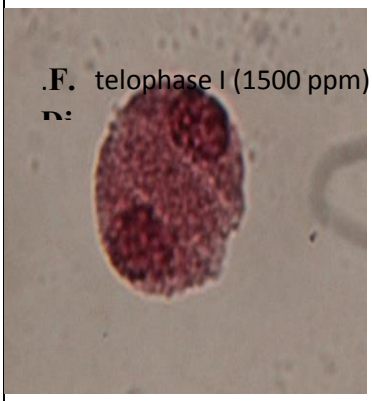
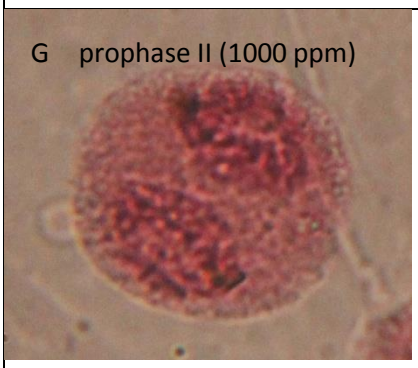
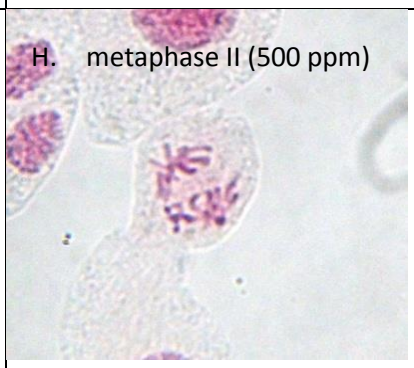
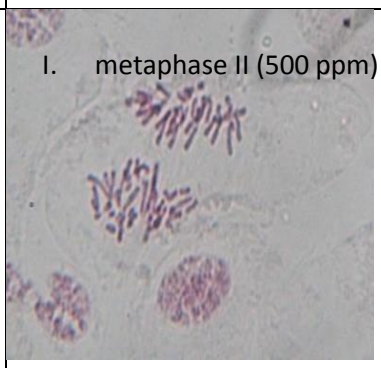


Figure Plate 7. Various abnormalities recorded in *A. ascalonicum* at different concentrations.

A. Anaphase I (1500 ppm) 	B. Anaphase I (1500 ppm) 	C. telophase I (1000 ppm) 
D. Anaphase I bridge (1500 ppm) 	E.Anaphase I breaks (1500ppm) 	.F. telophase I (1500 ppm) 
G prophase II (1000 ppm) 	H. metaphase II (500 ppm) 	I. metaphase II (500 ppm) 

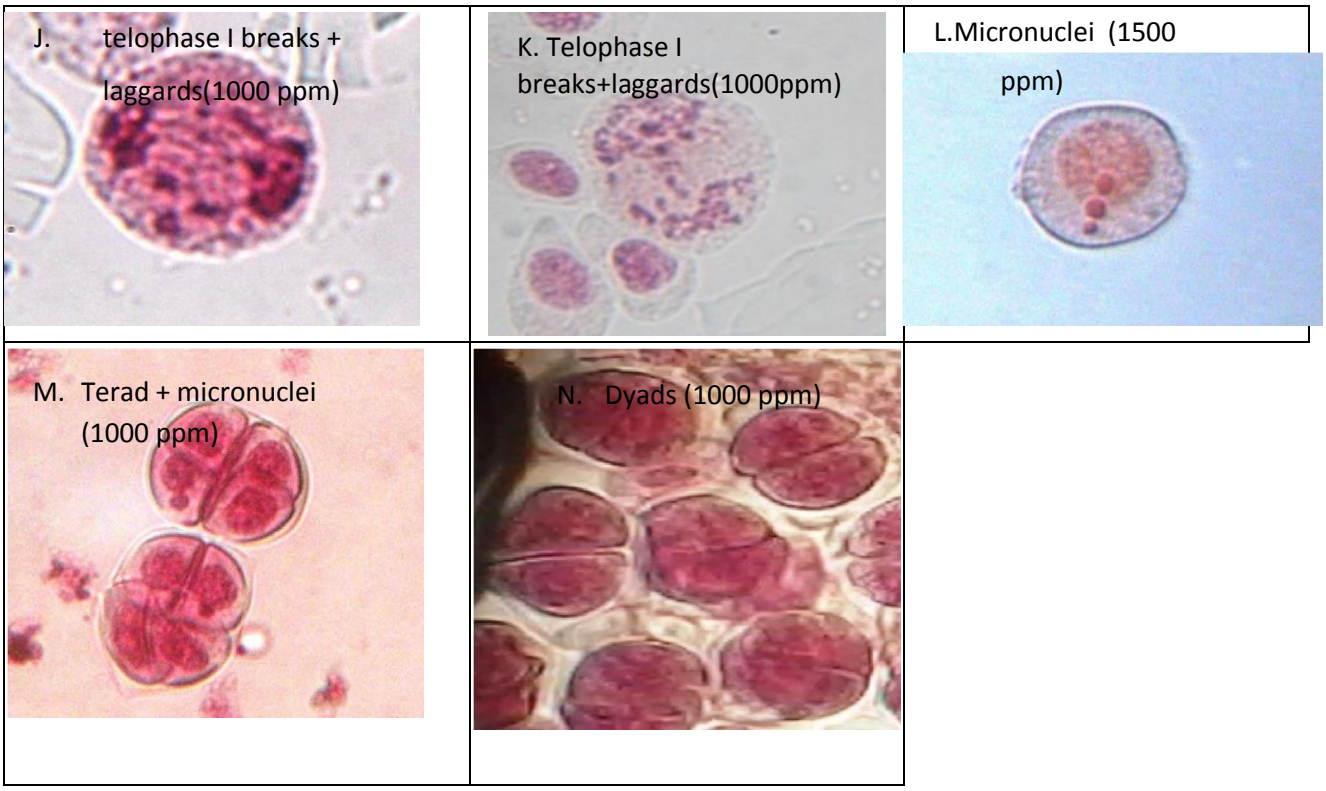


Figure Plate 8: various abnormalities recorded in *A. hookeri* at different concentrations of SDS

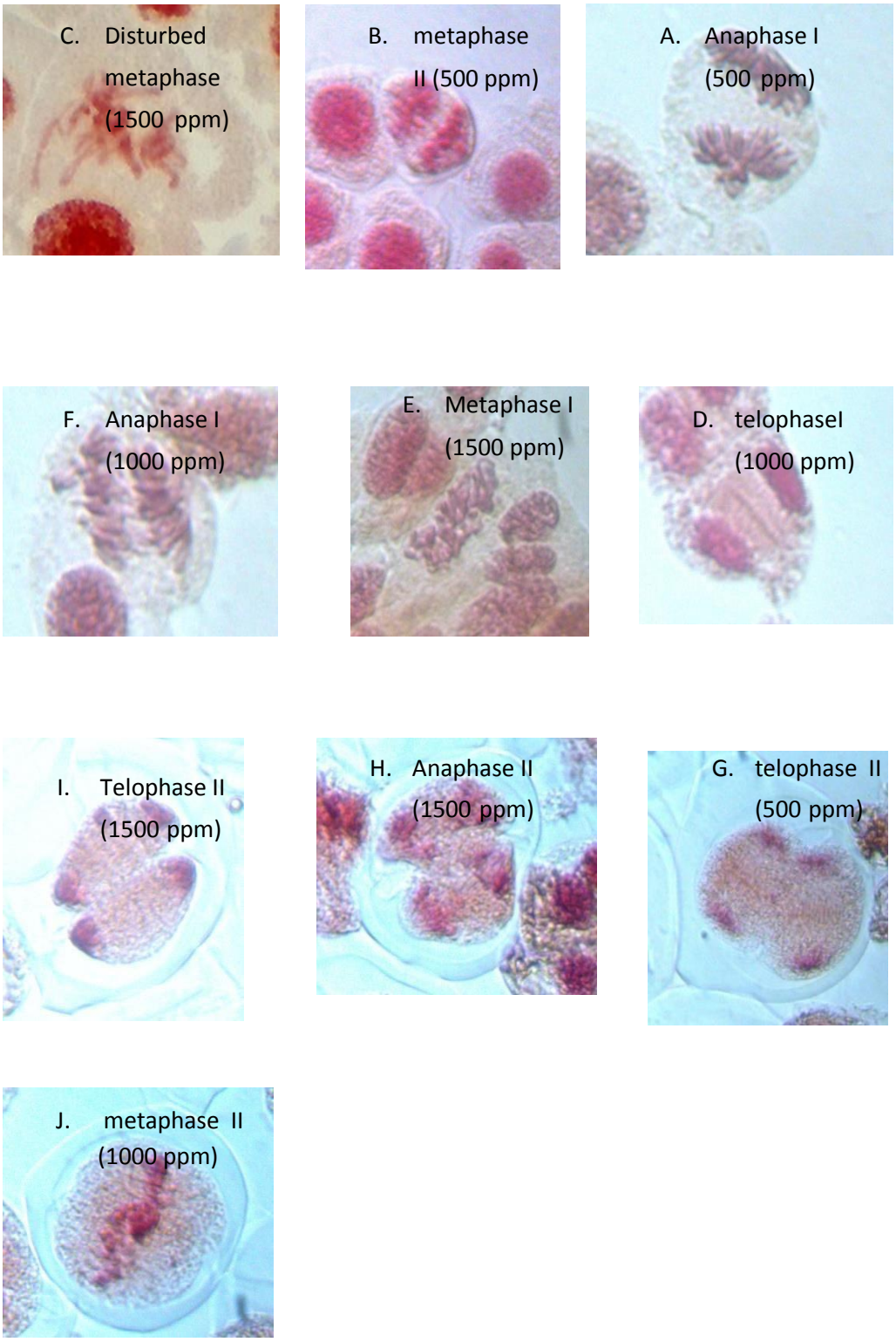


Figure Plate 9: Various abnormalities recorded in *A. tuberosum* at different concentrations of SDS

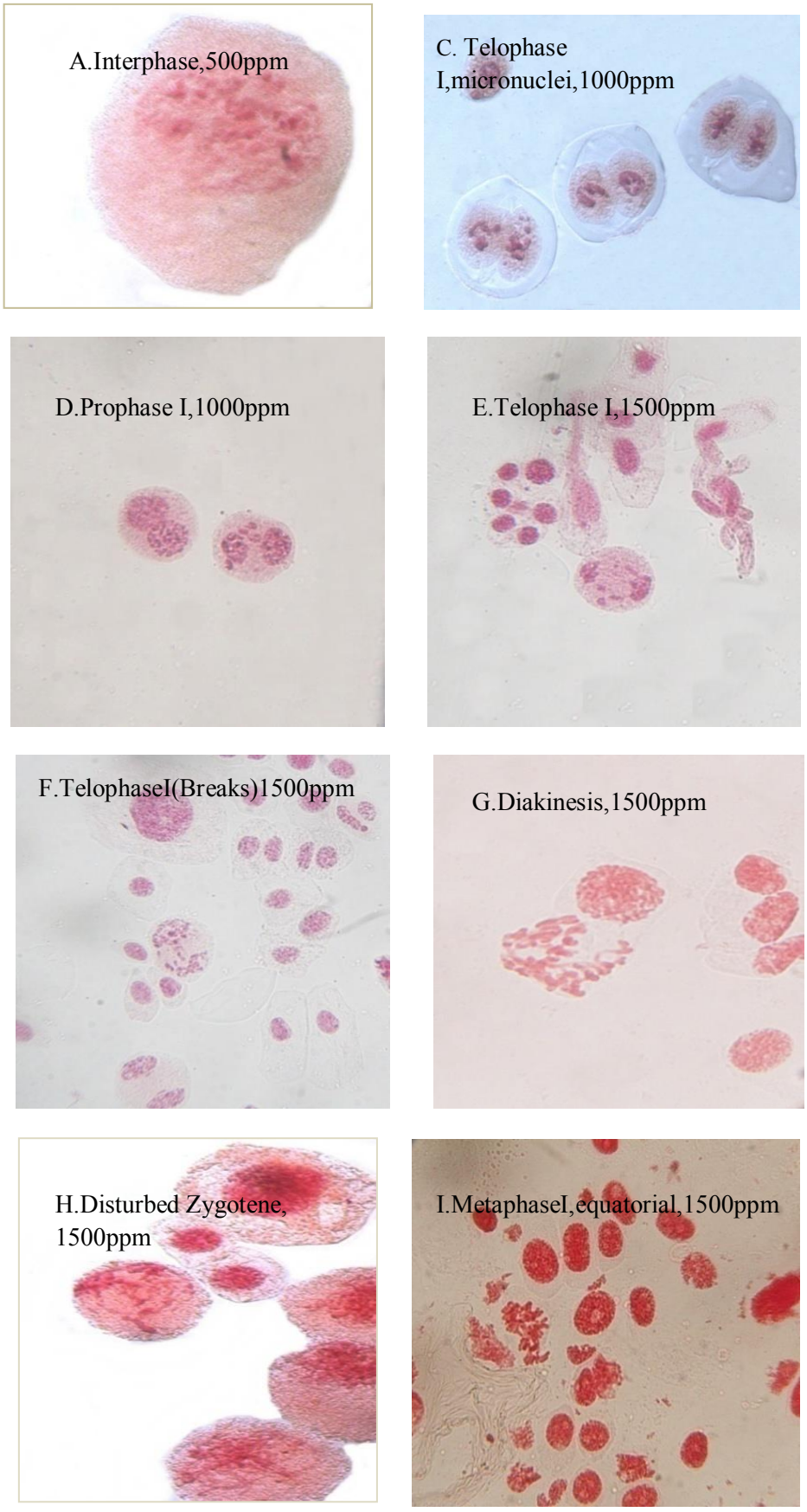
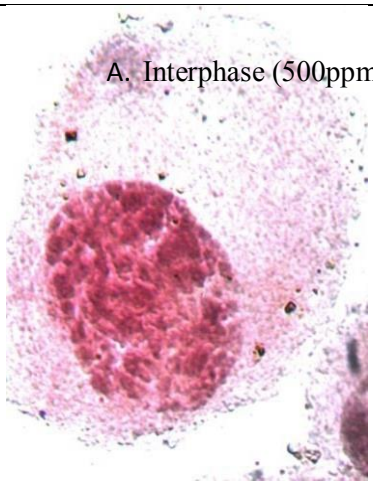
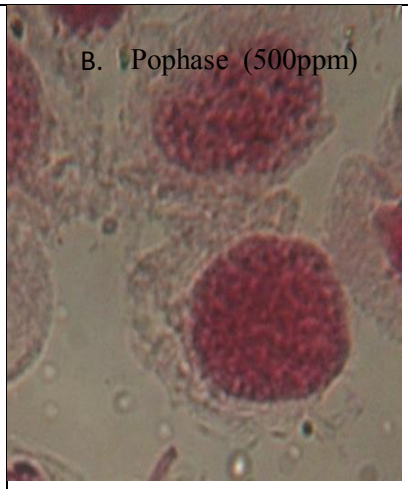


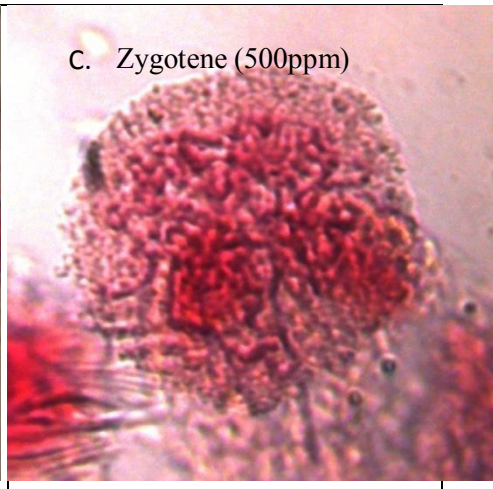
Figure Plate 10. Various abnormalities recorded in *A.porum* at different concentrations of SDS



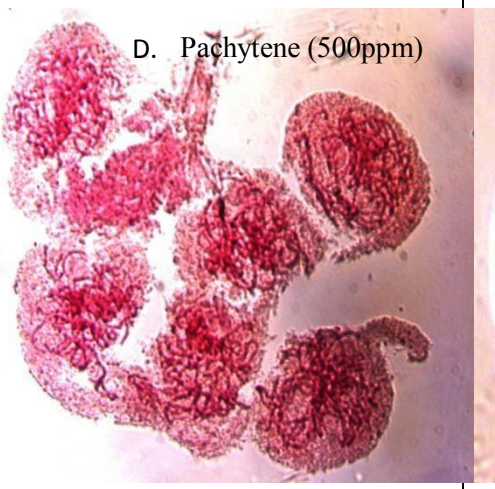
A. Interphase (500ppm)



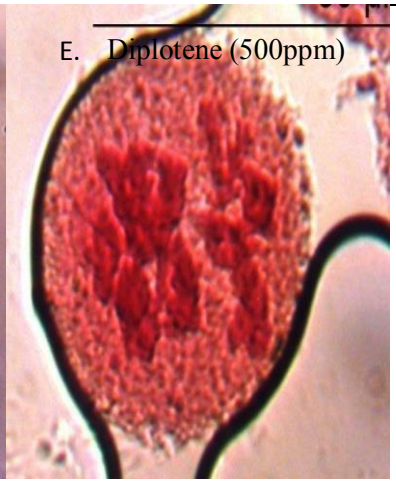
B. Pophase (500ppm)



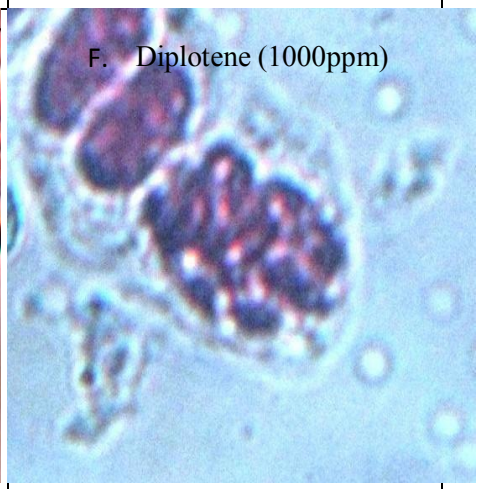
C. Zygotene (500ppm)



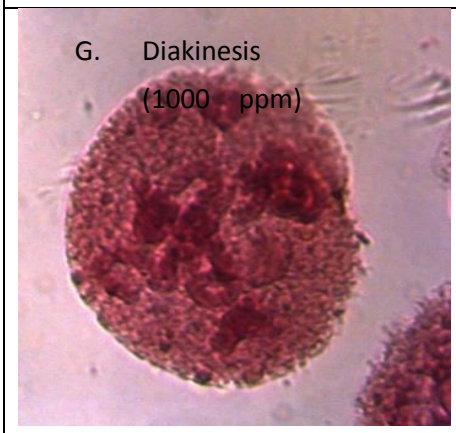
D. Pachytene (500ppm)



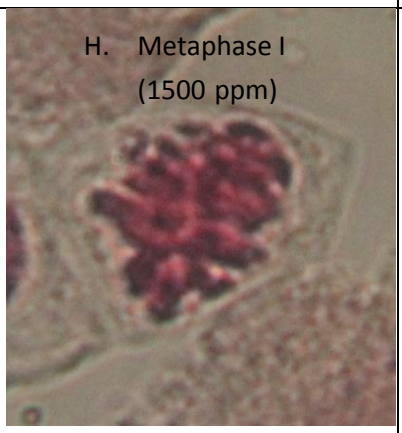
E. Diplotene (500ppm)



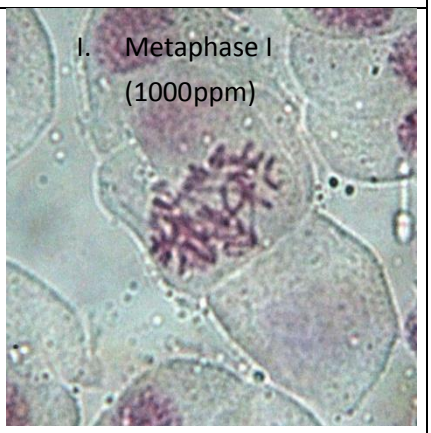
F. Diplotene (1000ppm)



G. Diakinesis (1000 ppm)



H. Metaphase I (1500 ppm)



I. Metaphase I (1000ppm)

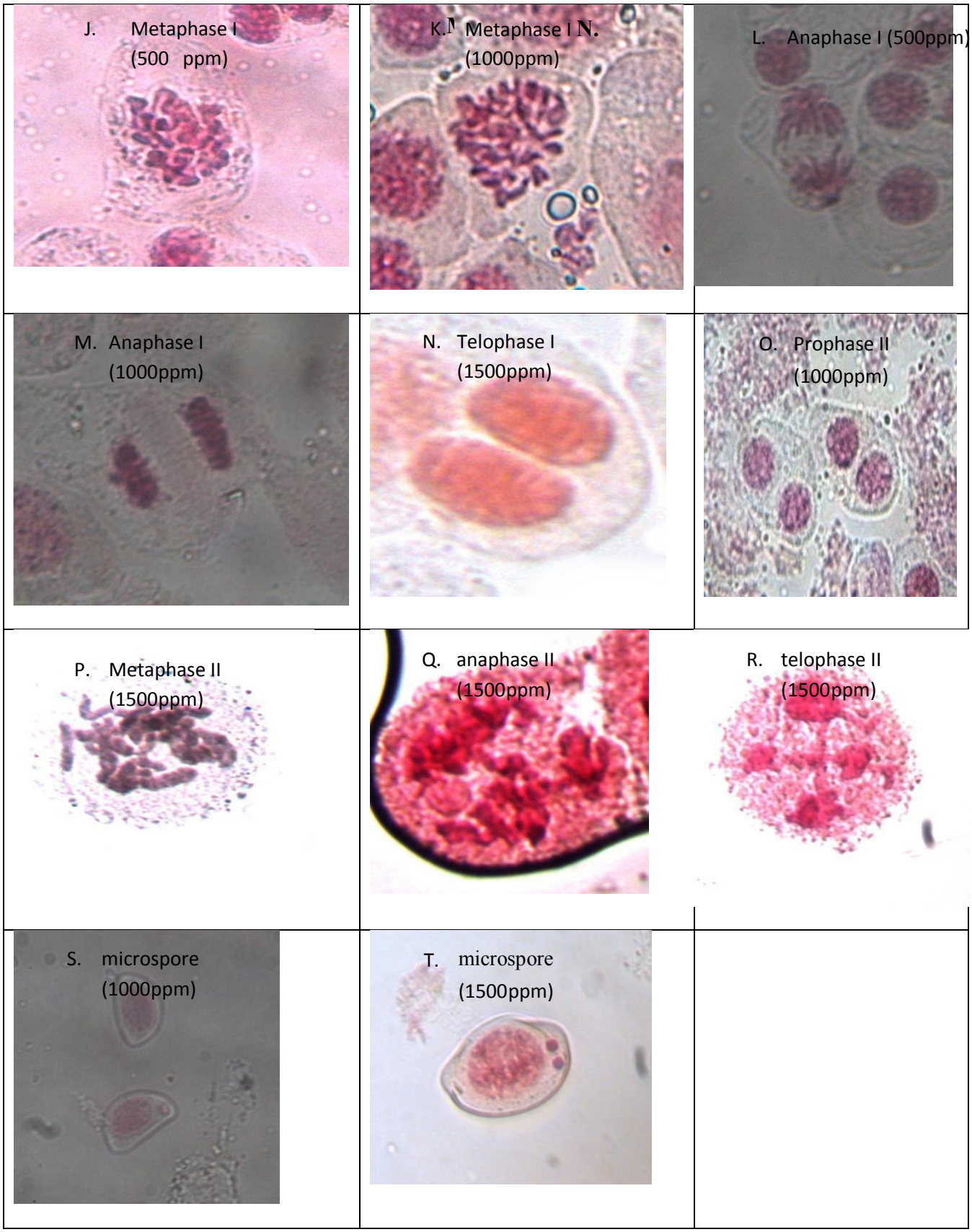


Figure Plate 11: Various abnormalities alities observed in *A. chinense* at different concentration of SDS

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1. Kumar, S., Kirha, T.J. and Thonger, T. 2014. Toxicological effects of sodium dodecyl sulfate. *Journal of Chemical and Pharmaceutical Research* **6**(5): 1488-1492.
2. Kirha, T.J., Thonger, T. and Kumar, S. 2016. A Review on the Benefits of *Allium sativum* on Cancer Prevention. *Journal of Cancer Treatment and Research* **4**(5): 34-37. http:// doi: 10.11648/j.jctr.20160405.11
3. Kirha, T.J. and Kumar, S. 2017. Physiological and Clastogenic Effects of SDS in Certain *Allium* Species of Nagaland. *Journal of Chemical and Pharmaceutical Research* **9**(10):182-185.

Paper Presented in Seminars and Conferences

1. Sodium dodecyle sulphate genotoxicity in *Allium tuberosum* In: National Seminar on 'Inventory,Sustainable Utilization & Conservation of Bioresources' held at Nagaland University, Lumami on February 26- 27, 2016.
2. To study normal mitotic stages and effect of SDS on meiosis in *Allium hookeri* Thw. In: National Seminar on 'Inventory,Sustainable Utilization & Conservation of Bioresources' held at Nagaland University, Lumami on February 26- 27, 2016.
3. Cytotoxicity of Sodium dodecyl Sulphat in *Allium chinense.*In: National Seminar on Advances in Biological Science Research, Department of Botany, Nagaland University, Lumami on February 28- March 01, 2017.
4. Physiological and Clastogenic effects of SDS in Certain *Allium* Species of Nagaland In: International Conference on Natural Resources Management and Technology Trends (ICNRM-17) Centre of Advanced Study, Department of Life Sciences, Manipur University on March 27-29, 2017.

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