A STUDY OF THE POTENTIAL GENOTOXIC EFFECTS OF SOME MEDICINAL PLANTS COMMONLY USED IN KENYA USING THE *SALMONELLA*/MAMMALIAN MICROSOME ASSAY AND THE *VICIA FABA* TEST SYSTEM

by

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DECLARATION

I, Anastasia Ngure Wairimu, hereby declare that this thesis is my original work and has not been presented for a degree in any other university. All sources of information have been acknowledged by means of references.

Anastasia Ngure Wairimu

This thesis has been submitted for examination with my approval as the university supervisor.

Professor H.N.B. Gopalan

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ABSTRACT

Several plant products such as the pyrrolizidine alkaloids, flavonoids, cycasin, quinones and bracken fern toxins have been reported to possess genotoxic properties in various test systems. However, crude drugs from plants, i.e., the complex mixture from which these drugs are extracted have been only scantily examined for their genotoxic activity. Such crude drugs are widely used in traditional medicine in various parts of the world.

In the current study, a number of plant extracts, namely; the fresh sap of *Aloe graminicola (A. graminicola)*, and the methanol extracts of *Annona senegalensis (A. senegalensis)*, *Centella asiatica (C. asiatica)*, *Maesa lanceolata (M. lanceolata)*, *Myrsine africana (M. africana)* and *Myrica salicifolia (M. salicifolia)* which are commonly used as Kenyan traditional medicine were investigated for their potential genotoxic effects in the Ames/*Salmonella*/mammalian microsome assay and in the *Vicia faba (V. faba)* test system.

The results of this study revealed that the *Salmonella* test is an indispensable tool in the detection of mutagens in crude drugs. In general, metabolic activation with the liver microsomal fraction (S9) increased mutagenic activity of these extracts.

However, evidence of deactivation by S9 of the methanol extracts of *A. graminicola* in TA97a and *A.senegalensis* in TA100 was recorded. The methanol extract of *A. senegalensis* was found to be mutagenic in all the histidine mutants (TA97a, TA98, TA100, TA102, and TA104), but relatively small number of revertants were recorded in all tester strains (the highest being a mean of 549 revertants/plate/1000 ug in TA104 (+S9) compared to $1\text{-}2^8$ cells plated in the mutagenicity assay). This means that histidine which may be present in crude drugs could not have influenced positive results in the extract of *A. senegalensis*.

The plant extracts were found to induce reverse mutations in most of the tester strains. However, TA104, a strain that contains a nonsense mutation (AT) at the critical site of reversion, yielded the highest number of revertants with most of the plant extracts. The analogous strain TA102 detected some of these mutagens but not as effectively as TA104. The greater reversion of TA104 as compared to TA102 suggests that the deletion of the *uvr*B gene facilitates better detection of crude drugs as mutagens.

One of the limitations of the Ames test for the detection of mutagens in crude drugs was the reduction in number of revertants at higher doses. In two plants, *M. africana* and *M. salicifolia*, toxic

occurring in the roots allowed to recover could have been induced in G_1 or S phase of the cell cycle.

The fresh extract of *A. graminicola* produced micronuclei in roots allowed to recover for 20 hours. This gives evidence on the possibility of loss of genetic material in cells exposed to this extract.

The methanol extract of *M. lanceolata* increased the size of nucleoli in roots allowed to recover for 20 hours. Nucleolar enlargement (nucleolar oedema) could be an artifact as a result of subjecting the cells to unphysiological conditions. Statistical analysis revealed that there was no relationship between size of nucleoli and dose of treatment.

From the results presented in this study, several plant extracts used in Kenyan traditional medicine are likely to contain mutagenic (genotoxic) ingredients. Some are activated by mammalian microsomal enzyme fractions while certain others are deactivated to some extent. Further studies with purified extracts and other test systems are needed before recommendations regarding their continued use in traditional medicine can be made.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1. Introduction

Genotoxicity is a concept which means "toxicity to the genome" (Hofnung and Quillardet, 1984). Genotoxic agents may cause long term deleterious effects in the genetic material when encountered at subtoxic concentrations. For example, a genotoxic agent may cause a deleterious mutation in the germ cell or a germ cell precursor and this effect may be transmitted to the offspring and their descendants. Eventually there is likely to b an accumulation of heritable traits in the gene pool. Genotoxic agents may be teratogenic, meaning that they may interfere with normal embryonic development and offspring born by affected pregnant mothers may carry congenital malformations. When mutational damage occurs in the genome of somatic cells, this may cause formation of neoplastic tissue which can become malignant, as in cancer.

Apart from mutagenesis, teratogenesis and carcinogenesi other irreversible effects brought about by genotoxic agents include cell death, phage-induction and chromosome breakage (Hofnung and Quillardet, 1984). A reversible effect caused by genotoxic agents is

exemplified by inhibition of cell division (Hofnung and Quillardet, 1986). Thus each of these end-points may be taken as an indication of genotoxicity.

Knowledge of genotoxicity can be traced back to the pioneering work of Muller (1927) who demonstrated the induction of point mutations in *Drosophila* using x-ray irradiation. Later, Auerbach (1949) demonstrated the effect of the chemical, nitrogen mustard, on the chromosomes of *Drosophila*. But during those early days chemical mutagenesis was only concerned with the chemistry of the genetic material. It is only within the recent past that geneticists have expressed concern over the mutagenicity of both natural and man-made chemicals and this is because of the vast number of chemicals which are being introduced into the environment.

Approximately 4.5 million different chemical compounds are in every day use in form of food additives, pesticides, agrochemicals, therapeutics and cosmetics (Maugh, 1983). Out of these only a minor fraction has been tested for their potential to cause genetic damage. There is evidence that environmental factors contribute to cancer (Howe, 1975; Cairns, 1979) and congenital malformations (Kalter, 1971), and it is known that damage to DNA is a major cause of cancer (Ames, 1979; Doll, 1979), heart disease (Benditt, 1977) and many other human disorders (Arlett and Lehmann, 1978).

Chemicals cause genetic defects through point mutations, also referred to as gene changes, (Drake and Baltz, 1976), or through chromosomal anomalies as in aneuploidy (Sandhu *et al.*, 1986) and chromatid aberrations (Savage, 1976). But these lesions induced by chemicals or even radiations are not in themselves mutations, nor do they cause cancer directly because cells can repair such primary damage. Thus when there is an accurate repair (error-free), lesions are not manifested as mutations (Witkin, 1976; Kimball, 1978), whereas when there is an inaccurate repair (error-prone) the lesions are manifested as mutations. Genetic toxicologists are thus concerned with screening for the consequences of the mistakes made by the cell in an effort to correct damage induced by external agents (xenobiotics).

A high correlation between chemically induced carcinogenesis and mutagenesis has been reported (McCann *et al.*, 1975a; Purchase *et al.*, 1976). Direct evidence appears to support this relationship in human hereditary disorders such as xeroderma pigmentosum (XP), ataxia telangiectasia (AT), Fanconi's anaemia (FA), Bloom's syndrome (BS) and Cockyanes syndrome (CS). the cells of these individuals show an abnormal cellular response to DNA damaging agents (Arlett and Lehmann, 1978; Evans, 1980). For example; XP, BS and CS patients show high sensitivity to solar

radiation indicating a possible defect in repair of UV-induced damage. Such patients develop cancer leading to early death (Arlett and Lehmann, 1978).

Genotoxic agents can produce alterations that interfere with cell differentiation during embryonic development resulting in teratogenic defects but it has not been established whether all mutagenic compounds are also teratogenic (Kalter, 1971). Many individuals with birth malformations exhibit chromosomal aberrations in their cells suggesting that their cells may be more prone to mutational damage than those of normal individuals. It has been shown that ionizing radiations produce a higher frequency of chromosomal aberrations in AT cells than in normal cells (Taylor *et al.*,1976; Taylor, 1978). Chromosomes in cells of BS are more highly sensitive to the mutagen, ethylmethanesulfonate (EMS; Krepsinky *et al.*, 1978).

Teratogenesis is thought to be linked with cancer because it has been found that children with malformations have a high incidence of childhood cancer (Miller, 1977). Heart disease may be due to mutations. Atherosclerosis, a plaque in the coronary artery is probably due to monoclonal proliferation of cells stemming from a mutated cell (Benditt, 1977).

Thus, given that man is exposed to a bewildering array of chemicals, some of which increase the risk of cancer, birth malformations and heart disease, there is

an urgent need to screen and identify potential carcinogens, mutagens and teratogens with a view to minimize human exposure to such chemicals and reduce the risk.

Today, short term tests for the detection of potential mutagens and carcinogens are being incorporated in the package recommended for toxicological tests of chemicals by the major regulatory agencies, e.g. US Occupational Safety Administration (OSHA) for classification of chemical carcinogens in the work place, and by the US Environmental Protection Agency (EPA) for regulating chemicals. Other efforts are being made by the International Agency for Research in Cancer (IARC) under the auspices of the World Health Organisation (WHO) for the evaluation of carcinogenic risk of chemicals to man.

1.2. Review of literature

1.2.1. Short term tests for the detection of potential mutagens and carcinogens

The most direct method for the detection of human carcinogens is by population studies. But this method is expensive and has a low resolving power. Only a handful of chemicals are known to be carcinogenic to man. Most of these have been detected following studies of workers occupationally exposed to such chemicals. The classic example is soot which has been known for over 200 years to cause scrotal cancer in young chimney sweeps (Pott, 1963). More recent examples are asbestos, 2-naphthylamine and vinylchloride (I.A.R.C. Monographs: 1973, 1974 and 1976) which produce rare cancers of the lungs, bladder and liver, respectively. In many cases difficulties arise in isolating the effects of individual agents since populations are usually exposed to multiple agents.

The alternative is to carry out long term carcinogenicity tests with laboratory mammals; but these have disadvantages. For example they are expensive; around $300,000 are needed for one chemical (Hollstein *et al.*, 1979). They are time consuming; a single test takes upto 3 years to complete (Hollstein *et al.*, 1979). Since only a relatively small number of animals can be examined (50 animals per treatment group; Tennant *et al.*, 1987) these tests are limited in sensitivity. Tests for heri-

table germline mutations take somewhat less time to complete but they require large numbers of animals making them very expensive. For example, the mouse specific locus test (Russel, 1951) takes about 8 months to complete, 10,000 - 50,000 progeny must be scored depending on the sensitivity of the test (Searle, 1975). This method is then unsuitable for testing large numbers of chemicals. Using animals to such a large scale is also being vigorously opposed by animal welfare bodies (Langrey, 1984).

A number of "short term" tests for detecting potential carcinogens and mutagens have now emerged. These tests have largely been developed to detect rodent carcinogens. The performance of these tests is primarily assessed using rodent carcinogenicity test results (Tennant *et al.*, 1987). About 100 of these tests are in more or less regular use (Hollstein *et al.*, 1979). They range from use of pro- and eucaryotic microorganisms, mammalian cells in culture, insects and plants. During development of these tests, it has become clear that no single test is capable of detecting all rodent carcinogens. Therefore, a battery of tests is needed so that the strength of one test can compensate for the inadequacies of another in detecting specific classes of mutagens. In this review, only the most widely used short term tests are discussed.

1.2.1.1. Tests using procaryotic microoganisms

Tests utilizing microorganisms are especially useful because cells grow rapidly and populations of treated cells are very large. Positive data is often very distinct from control values and tests are technically simple and inexpensive. In assays with microorganisms, a preparation of liver microsomes can be used *in vitro* to activate procarcinogens to ultimate carcinogens (Sugimura, 1979). Various short term tests utilizing procaryotic microorganisms have been reviewed recently (Hofnung and Quillardet, 1986). In this category only the inductest and the *Salmonella* tests are discussed.

E. coli K-12(λ): the inductest

Even before the structure of DNA had been established, Lwoff (1953) suggested that inducible lysogenic bacteria might become a good test for the detection of carcinogenic and anticarcinogenic activity. A bacterium is said to be lysogenic when it carries in a dormant form the DNA of a temperate phage which in this dormant form is called a prophage. One such phage is lambda (λ), which becomes a prophage when it is integrated in the DNA of certain lysogenic strains of *E. coli*. When lysogenic *E. coli* cells are subjected to any treatment that halts DNA replication, the prophage is induced. Its DNA loops

out of the bacterial chromosome, directs the synthesis of proteins, forming viral particles. A progeny of mature phages assemble which are later released by bursting out of the host cell (Devoret, 1979). This process is called lysogenic induction. In normal circumstances, the dormant stage of prophage is maintained by a repressor protein on the operator region and blocks the transcription of all genes except those that govern the biosynthesis of the repressor to keep the prophage dormant.

The use of prophage induction as a screening method to detect potential mutagens and carcinogens has been reviewed by Heinemann(1971). A tester strain, GY5031 has been developed by introducing into *E. coli* K-12 an *env* A mutation that increases permeability to many mutagens and an *env* B mutation that inactivates the excision repair system (Moreau *et al.*, 1976; Hollstein *et al.*, 1979). By combining the tester strain with a metabolic activation system, the inductest has become very effective for detecting many environmental mutagens and carcinogens. Procedures have been defined to test for both lysogenic induction and mutagenicity in the same cell (Moreau *et al.*, 1976). In the test for induction, induced lysogens form infective centres on a lawn of non-lysogenic indicator bacteria, and plaques are counted after overnight incubation on ampicillin plates. The tester strain GY5031 is ampicillin sensitive and the indicator strain

is ampicillin resistant. Ampicillin prevents non-induced lysogens from multiplying and releasing phage. To detect mutations, the same bacterial tester strain GY5031 is plated on lysogenic indicator cells. When forward mutations occur at the operator region of this prophage, it becomes insensitive to the repressor resulting in the formation of infective centres on a lawn of lysogens. An enzyme assay to detect prophage induction has been developed in a strain that biosynthesizes galactokinase during lysogenic induction (Levine *et al.*, 1978). The assay takes only 6 hours to complete.

The *Salmonella typhimurium* (*S. typhimurium*)/ mammalian microsome test

The *Salmonella* assay or the Ames test (Ames, 1971) is the most commonly used test utilising bacteria. This test has recently been reviewed by Kier *et al.*, (1986) and Hofnung and Quillardet (1986). The *Salmonella* test is now recognised as the standard test for detecting mutagens and carcinogens and has been used for more than a decade in laboratories all over the world (Maron and Ames, 1983).

The test was first validated in a study of 300 chemicals most of which were known carcinogens (McCann *et al.*, 1975a; McCann and Ames, 1976). Their study showed that there is a high correlation between carcinogenicity and

mutagenicity; 90% of all carcinogens studied were mutagenic and *vice versa*. The test was subsequently validated in studies of Purchase *et al.*, (1976) who compared the *Salmonella* test with 5 other short term tests. They found that out of 58 known carcinogens, 91% showed mutagenic activity and that 93% of 62 non-carcinogens studied were non-mutagenic, but there was considerable overlapping of the chemicals studied so that this correlation is currently estimated at 83% (Ames and McCann, 1981).

The introduction of S9 fraction from rat liver induced with polychlorinated biphenyls has made the Ames test even more effective because the chemical tested is subjected to mammalian metabolic processes (Ames *et al.*, 1973b). This is especially effective in the conversion of pro-carcinogens to ultimate mutagens. Recently, Maron and Ames (1983) have included modifications of the original methods (Ames *et al.*, 1975) to allow flexibility and improve the sensitivity of the test. Earlier, Cheli *et al.*, (1980), had pointed out both inter- and intra- laboratory variations in the Ames test results even for direct acting mutagens like N-methyl-'N'-nitrosoguanidine (MNNG) in tester strains TA100 and TA1535.

In the *Salmonella* test, a set of histidine dependent mutants are used for mutagenicity testing. Tester strains contain specific types of mutations in the histidine operon

(Table. 2) rendering them unable to synthesize enzymes required for the biosynthesis of histidine, a necessary component of proteins. As a result of this deficiency, the strains are unable to grow in minimal medium (medium containing minerals and glucose only) unless the medium is supplemented with histidine. These his auxotrophs on rare occasions can undergo a reverse mutation which restores the DNA to normal coding sequences for the needed enzyme as in the his$^+$ prototrophs (wild type). Thus, these strains detect mutations that are capable of inducing auxotrophs to become prototrophs. They can also detect compounds that act as suppressor mutagens which also restore gene function.

Trace amount of histidine is added to the minimal medium and this allows examination of bacterial background lawn which determines whether the test compound is toxic or not (Maron and Ames, 1983). If the test chemical is toxic the background is usually thin and sparse as compared to the control. This means that the available histidine resulting from the death of spontaneous revertants allows growth of lawn to visible tiny colonies which are not true revertants.

True revertants are counted against the background lawn after 2 days of incubation at 37°C. In addition to the histidine mutation the tester strains also contain other mutations that enhance their ability to detect mutagens. One such mutation is called "deep rough"(*rfa*)

which causes partial loss of the polysaccharide barrier that coats the surface of bacteria and increases permeability to large molecules such as benzo(a)pyrene which do not normally penetrate bacterial cells (Ames *et al.*, 1973a).

The tester strains contain another mutation *uvr*B which is a deletion of the gene coding for DNA excision repair system. This mutation greatly enhances sensitivity to many mutagens (Ames *et al.*, 1971; Ames *et al.*, 1973a). Creation of this *uvr*B mutation deletes the *bio* gene and as a consequence, these bacteria also require biotin in the external medium.

The primary tester strains (designated TA97, TA98, TA100 and TA102) contain a plasmid pKM101, which is an autonomous DNA fragment that makes DNA repair more error-prone. This plasmid is detected in tester strains by their ability to confer resistance to the antibiotic, ampicillin, in all tester strains. The plasmid enhances the sensitivity of tester strains to many mutagens (McCann *et al.*, 1975b; Levin *et al.*, 1982b).

The genotypes of the various TA strains used for mutagenicity testing have been described by Maron and Ames (1983). The hisG46 mutation in TA100 and TA1535 is in the hisG gene coding for the first enzyme in histidine biosynthesis (Ames, 1971). In this strain, a leucine codon (CTC) has been mutated to a proline codon (CCC;

Barnes *et al.*, 1982). TA1535 and its R-derivative TA100 detect mutagens that cause base-pair substitutions like transitions (GC-AT) and transversions.

The hisD3052 mutation in TA1538 and its R-derivative TA98 is in the gene coding for histidinol dehydrogenase. TA98 and TA1538 detect various types of frameshift mutagens. Frameshift mutagens can stabilise the shifted pairing that often occurs in repetitive sequences or "hot spots" of the DNA resulting in a frameshift mutation which restores the correct reading frame for histidine biosynthesis. The hisD3052 mutation has the sequence

-CGCGCGCG-
-GCGCGCGC- i.e., 8 repetitive -GC- residues near the site of the frameshift mutation in the hisD gene (Isono and Yourno, 1974).

A new frameshift strain TA97 replaces TA1537 which was previously included in the standard set of tester strains (Ames, 1975). This new tester strain has an additional cytosine at the site of hisD6610 mutation (Levin *et al.*, 1982a). The specificity of TA97 is similar to that of the hisC3076 in TA1537 but differs from it in possessing a second hot spot of alternating -GC- base pairs near the run of cytosines. It is sensitive to some of the mutagens that revert TA98 and TA1538 but not TA1537. However, TA97 has low levels of viability in overnight cultures, thin background lawn and pin point colonies on minimal agar plates (Ames, personal communi-

cation). Thus TA97 has been reconstructed to TA97a which carries a hisO1242 mutation.

Another tester strain is TA102 which does not contain uvrB mutation because it was constructed to detect oxidative mutagens which require an intact repair system (Levin *et al.*, 1982b). This strain has a multicopy plasmid, PAQ1, which carries a his ochre mutation, TA, at the site of reversion.

Another strain TA104 has the same ochre mutation as TA102 at the critical site of reversion but this mutation is on a chromosome rather than on a plasmid. The hisG428 mutation in TA102 and TA104 has the sequence AGAGCAACTAAGAGC whereas the wild type has the sequence AGAGCAAGCAAGAGC (Levin *et al.*, 1982b).

1.2.1.2. Tests using eucaryotic microorganisms

Three fungi have been used extensively to screen potential mutagens and carcinogens: the yeast, *Saccharomyces cereviasiae* (*S. cerevisiae*, Mortimer and Manney, 1971; Zimmermann, 1973; Zimmermann *et al.*, 1984; Resnick *et al.*, 1986) and the molds, *Neurospora crassa* (*N. crassa*, DeSerres and Malling, 1971; Brockman *et al.*, 1986) and *Aspergillus nidulans* (*A. nidulans*, Kafer *et al.*, 1976; Kafer *et al.*, 1982;

Scott *et al.*, 1982).

The principal advantage of tests using eucaryotic microorganisms is that they are the simplest and the most rapid tests available for screening several kinds of mutagenic and chromosomal effects not detected in procaryotic microorganisms. These include mitotic crossing over, i.e. the reciprocal exchange between homologous chromosomes. If the cell is heterozygous in this region, division can result in non-identical daughter cells that are phenotypically distinct and homozygous at the recombination site. In the most commonly used tester strains either the colony colour or loss of a growth requirement is the phenotypic change observed.

Another event easily scored in fungi is gene conversion. This is also a recombination event, but in contrast to mitotic crossing over, it involves the non-reciprocal transfer of a small segment of DNA and convertants possess restored wild-type genotype rather than two homozygous recessive alleles at the recombination site. Another mutational event scored in fungi is mitotic non-disjunction. If homologous chromosomes fail to separate during mitosis, two distinct daughter cells are formed; one is trisomic and the other is monosomic for that chromosome. The monosomic cell will express phenotypically any allele that is heterozygous in the parent cells and this can readily be

detected when appropriate tester strains are used. Non-disjunction during meiosis also results in aneuploidy; one gamete is disomic while the other one is nullisomic and not viable.

Fungi, unlike bacteria, contain some P-450 mixed function oxidases. Some carcinogens are then active in fungi without need for exogenous metabolic activation (Callen and Philpot, 1977).

One of the principal limitations of fungi as test organisms is their relative inefficiency in detecting pro-carcinogens even after addition of exogenous metabolic system (Simmon, 1979). For example, 101 carcinogens and pro-carcinogens were tested for their ability to induce mitotic recombination in *S. cerevisiae* strain D3. All 21 ultimate carcinogens were positive. However only 17 of the 48 pro-carcinogens tested were recombinogenic. All these chemicals were tested with and without metabolic activation (Simmons, 1979). Most aromatic amines were inactive with the exception of N-acetyl-2-amino-acetylfluorene (N-acetyl-2-AAF). Possibly, this difficulty is due, in part to limited permeability; a high RNA to DNA ratio and the presence of non-nuclear DNA which might serve as a sink for reactive intermediates, could also affect sensitivity of these tests (Hollstein *et al.*, 1979). Tests with the most commonly used fungi are briefly

described below.

Yeasts

Saccharomyces and to a lesser extent *Schizosaccharomyces* are the yeasts most commonly used in mutagenicity assays. Tests using yeasts have been reviewed by Zimmermann (1973), in an IARC monograph (1980) and Zimmermann (1984). Most tester strains detect genetic changes in specific regions of the genome: (i) mitotic crossing over (*S. cerevisiae* strain D3, D5 and D8); (ii) gene conversion (*S. cerevisiae* D4); or (iii) point mutations at loci that alter colony colour (*S. cerevisiae* *ade*1 and *ade*2 and *Saccharomyces pombe* p1).

Many yeast assays are designed to detect those genetic events in the region of the genome controlling adenine biosynthesis. Various alterations along this pathway will cause accumulation of a red pigment (resulting in the formation of red colonies), while other changes can block its formation, resulting in the formation of white colonies. An exogenous S9 mix is frequently added in assays using *Saccharomyces* (Hollstein *et al.*, 1979).

Molds

A. nidulans

Haploid strains are used to study forward and reverse

mutations in *A. nidulans* and the haploid tester strains have been combined into diploids for the detection of mitotic crossing over (Kafer *et al.*, 1976; Kafer *et al.*, 1982; Scott *et al.*, 1982).

The diploid heterozygous tester strains P and P1 do not grow on a medium containing p-fluorophenylalanine but if mitotic crossing over or non-disjunction has occurred, these strains grow. Colony colour markers in the strains permit a distinction to be made between p-fluorophenylalanine resistant colonies arising from mitotic recombination and mitotic non-disjunction. Pale green colonies are mitotic recombinants, while dark green colonies are due to mitotic non-disjunction (Kafer *et al.*, 1976).

N. crassa

N. crassa exists predominantly in the haploid phase, but can function genetically as a diploid by the formation two- component heterokaryons (pseudodiploid cells) which have been used to detect point mutations and small deletions (De Serres and Malling, 1971; Brockman *et al.*, 1986)

Foward mutations in strain *ad* 3A and *ad* 3B loci in heterozygotes cause the accumulation of a purple pigment, and mutations are detected by change in colony colour from white to purple. Reverse mutations can be

detected in two adenine requiring tester strains N23 which detect base-pair substitutions and N24 which detects frameshift mutagens using the spot, plate and suspension methods (Ong, 1978).

1.2.1.3. Tests using insects

Insects have been used extensively in mutagenicity testing. Most studies utilising insects have been carried out with *Drosophila melanogaster* (Abrahamson and Lewis, 1971; Vogel and Sobels 1976; Valencia *et al.*, 1982) and to a lesser extent with the wasp, *Habrabracon* (Smith and von Borstel, 1971) and silk worm, *Bombyx mori* (Tazima and Oniaru, 1974).

The sex linked recessive lethal (SLRL) test in *Drosophila melanogaster*

The SLRL test is the most commonly used test using *Drosophila* and the test procedure has been outlined by Würgler *et al.*, (1977) and Brusick (1980). The SLRL test is designed to detect point mutations in a large part of the *Drosophila* genome.

Recessive lethals are a heterogeneous class, they comprise point mutations (foward mutations and deletions) and both small and large rearrangements (Auerbach, 1962). Two generations are required for the detection of recessive lethals on the X-chromosome which represents about

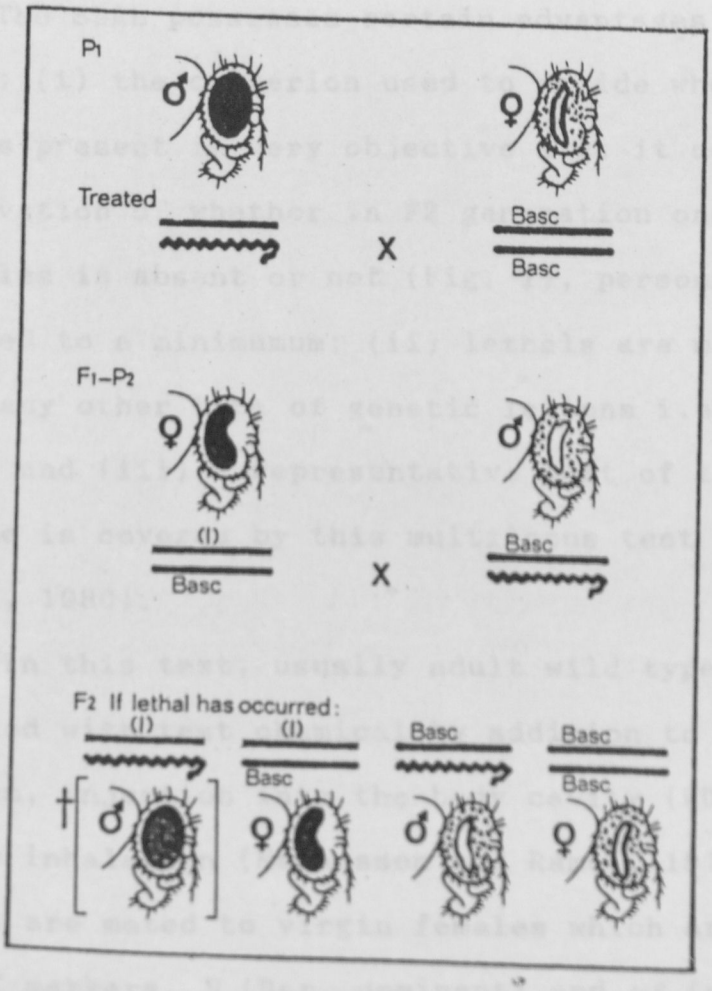


Fig. 1 The sex linked recessive lethal (SLRL) test in *D. melanogaster* (after Vogel and Ramel, 1980).

† = Phenotypic class absent

20% of the entire genome (Vogel and Ramel 1980).

The SLRL possesses certain advantages over other tests: (i) the criterion used to decide whether a mutation is present is very objective e.g. it consists of the observation of whether in F2 generation one entire class of males is absent or not (Fig. 1), personal bias is thus reduced to a minimumum; (ii) lethals are much more frequent than any other type of genetic lesions i.e. viable mutations; and (iii) a representative part of the *Drosophila* genome is covered by this multilocus test (Vogel and Ramel, 1980).

In this test, usually adult wild type males are treated with test chemical by addition to the nutrient medium, injection into the body cavity (Würgler *et al.*, 1977) or by inhalation (Magnusson and Ramel, 1976). The treated flies are mated to virgin females which are homozygous for the X markers, *B* (Bar, dominant) and w^a (white-apricot, recessive), which affect the shape and colour of eyes, respectively. Further the *basc* X-chromosome carries several inversion which prevents crossing over. Thus, the marker genes serve to distinguish "treated" (paternal) from "untreated" (maternal) chromosomes.

The F2 generation splits into four genotypes that can be identified by their different phenotypes, it is possible to distinguish the classes of flies which carry copies of a treated chromosome (Fig. 1). If a recessive

lethal mutation is induced in an X-bearing germ cell of the treated P1 male, all of the somatic cells of the resulting F1 female are heterozygous for for this mutation and 50% of the eggs will carry it. If a lethal mutation is induced, 50% of the F2 males will be hemizygous for it and will therefore die. This can be seen only if pair mating is done in the F1 generation. The main disadvantage in SLRL test is that it is very lengthy taking about one month to complete.

1.2.1.4. Tests using mammalian cells *in vitro*

Tests for mutations in cultured mammalian cells have been reviewed by Chu (1971), Arlett (1977), O'Neill *et al.*, (1977) and Hollstein *et al.*, (1979). Several mammalian cell types (both rodent and human) can be used in short term tests (Hollstein *et al.*, 1979; Kuroki *et al.*, 1980). Short term tests using mammalian cells are complementary to tests using procaryotic and eucaryotic microorganisms. Several carcinogens, such as natulan, 1,1-dichloro-2,2-bis(*para*-chlorophenyl)ethylene (p,p'-DDE) and diethylstilbestrol, which were negative in the *Salmonella*/microsome assay, are reported to be mutagenic in mammalian assays (Clive *et al.*, 1979).

One limitation of mammalian cells in culture is that although it is possible to demonstrate a dose response phenomena (of mutagens and carcinogens), often the number of mutational events scored is quite small

compared to pro- and eucaryotic microorganisms. In addition, mutation induction is frequently not observable until significantly toxic concentrations are reached and that dose response is over a very narrow range of concentrations (Hollstein *et al.*, 1979).

1.2.1.5. Tests using plants

Plants have been used in the research that has served to establish the principles of genetics and cytogenetics. They have been used to demonstrate the increase in frequency of mutations and chromosome aberrations induced by ionizing radiations (Mather, 1934; Sax, 1941) and to demonstrate mutagenicity of chemicals (McLeish, 1952; Smith and Lofty, 1955).

Mutagenicity and clastogenicity assays utilising plants to detect and/or monitor genotoxins in the environment offer several advantages compared to other assays utilising pro- or eucaryotic microorganisms. For example, plants are eucaryotic organisms whose chromosomal morphology and chemistry are similar to that of mammals. They have been shown to respond to mutagens in a manner similar to other eucaryotic organisms (Constantin and Owen, 1982). Certain species of plants are both sensitive to mutagens and have got a short life cycle (Ehrenberg, 1971; Kihlman, 1971; Ma, 1981).

Plants are uniquely adapted for *in situ* studies

and tests with plants are relatively cheaper as compared to procaryotic and mammalian cell assays. Assays that use plants have been extensively reviewed: *Allium* (Grant, 1982); *Arabidopsis* (Redei, 1982); *Tradescantia* (Ma, 1982); *Glycine* (Vig, 1982); *Hordeum* (Constantin and Nilan, 1982); *Vicia* (Ma,1982); and *Zea* (Plewa, 1982). The most commonly used bioassay utilizing plants involve the use of root meristems.

Root meristems

Root tips from various plant species have been widely used for the study of induced chromosomal aberrations. The most commonly used are those of *Vicia faba* (*V. faba*; broad bean, horse bean or field bean) and various species of *Allium* (Kihlman, 1971). Due to their low chromosome numbers and large size, availability all the year round, and the fact that they are easily handled and cultivated, these materials are favoured for cytological studies (Kihlman, 1971). In this review only *V. faba* is discussed.

V. faba (V. faba) cytogenetic studies

V. faba is a popular cytological, and radiobiological material not only because of the above mentioned reasons but also because its physiological reactions towards external agents is well known

(Kihlman, 1966).

Root tip cells of *V. faba* contain 5 pairs of equally long chromosomes with subterminal centromeres (S-chromosomes) and a pair with median centromere (M-chromosome). The M-chromosome pair is twice as long as the S-chromosome (Fig. 2). The M-chromosome bears a heterochromatic segment near the end of the non-nucleolar arm (Evans and Bigger, 1961). Heterochromatin is also found in the middle of the long arm of most of the S-chromosomes and chemically induced breaks are usually located in this region (Kihlman, 1971).

The duration of mitotic cycle in *V. faba* varies from 18-22 hr at 19°C (Kihlman, 1971). Evans and Scott (1964) calculated duration of the various mitotic stages as follows: G1 (4.9 hr); S (7.5 hr); G2 (4.9 hr.); and mitosis (2hr). The interphase occupies 17.3 hr.

Induction of chromosomal aberrations in *V. faba* is dependent on: (1) structure and dose of chemical; (2) stage of cell cycle at time of treatment; (3) p^H; (4) temperature; and (5) oxygen tension of the treatment solution. Thus methylated oxypurines such as 8-methoxycaffeine, 8-ethoxycaffeine and 1,3,7,9 -tetramethyluric acid produce subchromatid exchanges in prophase and chromatid exchanges in G2 but not in

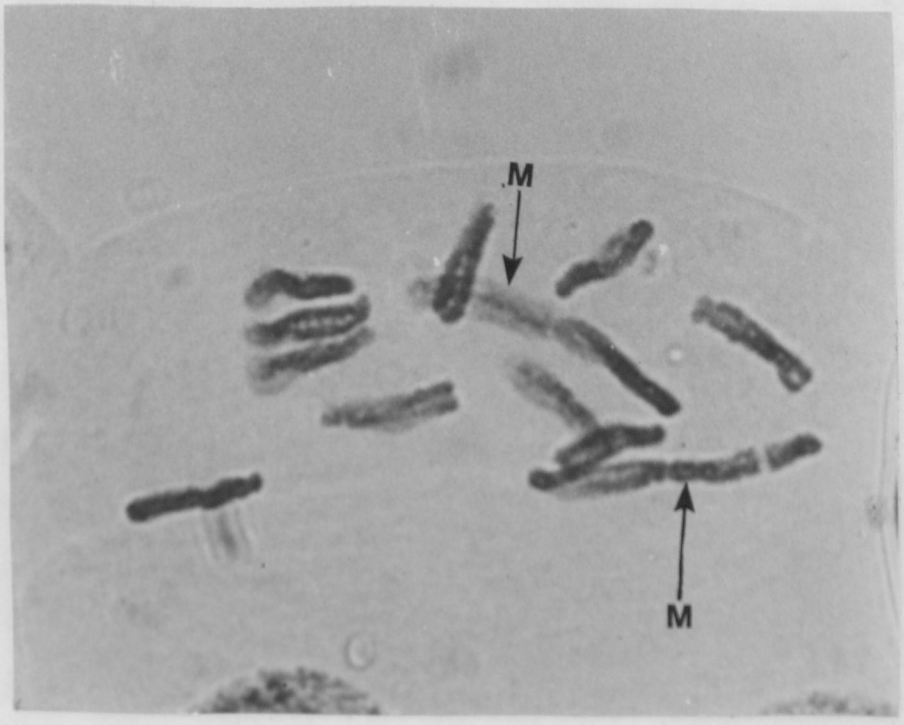


Fig. 2 A *V. faba* root meristem cell showing the normal diploid chromosome complement in metaphase. Note the 5 pairs of S-chromosomes and the satellited M-chromosome pair (×2200).

other stages (Kihlman, 1966; Kihlman, 1975). Alkylating agents like tris(1-azilidinyl)-phosphate (TEPA) and related compounds are active at all stages of the cell cycle but the aberrations they cause are dependent on DNA synthesis (Kihlman, 1971; Kihlman and Andersson, 1984). Thus, only cells exposed during G1 or S show aberrations in the first mitosis after treatment. Antibiotics such as phleomycin and bleomycin are active at all stages of the cell cycle and produce chromatid, subchromatid and chromosome aberrations in prophase, G2, S and G1, respectively (Kihlman and Andersson, 1984).

pH influences production of chromosome aberrations in *V. faba*. For example at pH 4.7 there were six times more aberrations than at pH 7.3 in the roots of *V. faba* treated with 2×10^{-4} M maleic hydrazide (Kihlman, 1956).

Chromosome breakage is also influenced by temperature. Maleic hydrazide was found to be more clastogenic at 25°C than at 3°C (Kihlman, 1966).

Oxygen tension also affects action of chemicals on chromosomes. For example, the number of isolocus breaks in chromosomes of *V. faba* after treatment with maleic hydrazide, potassium cyanide, methoxyphenylnitrosamine and 8-ethoxycaffeine increases with oxygen tension of the gas phase in the treatment solution (Kihlman, 1966).

1.2.2. Plants as source of drugs, carcinogens, mutagens and antimutagens

1.2.2.1. Plant drugs

The use of plants in the treatment of various diseases is universal. In Japan alone, 40,000 tons of crude drugs are consumed annually in the form of various chinese medicinal drugs (Morimoto *et al.*, 1982). In East Africa, native medicine has been practised for many centuries.

Humpreys (1982) has reviewed plant products which have succesfully been used in human medicine. For example, the digitoxins and digotoxins from *Digitalis purpurea*, foxglove, a common ornamental plant, have been used successfully to cure heart disease. The leucocristine and vincablastine alkoloids extracted from *Catharanthus roseus*, commonly known as Madagascar periwinkle, are used for the chemotherapy of leukemia. Cinchonines, cinchonidines, quinines and quinidines from the *Cinchona* tree have been used effectively in treating malaria. Gout can be controlled by use of colchicine from *Colchicum automnale*. Besides cures, plants have been utilized in the production of hormones. The mexican yam, *Discorea composita*, contains steroids used in the production of oral contraceptives (Humpreys, 1982).

Realising that plant products can cure diseases effectively, many research groups are now examining the vast number of untested plants. Often the first hint of

knowing whether a plant can cure comes from local herbalists though majority of them are reluctant in revealing their knowledge. In Kenya, this problem is being minimised because the government is encouraging local medicine men to practice their skills in co-operation with personnel from the Ministry of Health, and a department dealing with herbal medicines has been created at the Kenyatta National Hospital. At the moment local medicine men have formed an organisation called *Umoja wa Mitishamba* which engages in the promotion of herbal medicines.

1.2.2.2. Carcinogens and mutagens

Although plants are useful in the production of medicinal drugs, they can also be a source of carcinogens and mutagens (Nagao *et al.*, 1978).

Endogeneous secondary plant products are used by the same plant as a defense against fungal, bacterial and insect attacks as well as animal predation (Malinow *et al.*, 1982). Deleterious effects resulting from the consumption of plants are often discovered from sudden outbreak of toxicosis in livestock and poultry or even from frequent hepatomas or cancers in individuals of certain areas. In his comprehensive review, Berman (1958) discussed the possibility that plants used by

various people in form of cooking ingredients and folk medicine are responsible for human cancer in Africa and South East Asia. Plant products, therefore, are of economic importance because they may cause livestock and human disease. There is also a possibility that they contribute to an increase in mutation rate in the phytophagous animals (Clark, 1959). Some of the most important secondary plant products with toxic, carcinogenic and/or mutagenic activity are discussed below.

Pyrrolizidine alkaloids

The pyrrolizidine alkaloids (PAs) have been found to occur in 50 species of Compositae, Bignonaceae and Leguminosae (Bull *et al.*, 1968). These families include species which are used as medicinal herbs in East Africa (Kokwaro, 1976). PAs are highly toxic and are responsible for cancer in humans and liver disease in grazing animals (Tazima, 1974; Clark, 1976; Tazima, 1984). Experimental evidence indicates that these alkaloids are carcinogenic. For example, Schoental (1957) demonstrated severe liver lesions in experimental animals by the *Senecio* alkaloids, seneciphylline, seneconine and lasiocarpine. Other carcinogenic PAs are jacobine, monocrotaline, redelline, hydroxyserkinine and isatidine (IARC monograph, 1976). Petastanin, a PA

isolated from the flower stalks of *Petasites japonicus* which is used as a herbal remedy in Japan, has also been shown to be a hepatocarcinogen in rats (Hirono *et al.*, 1977).

The characteristic feature of chronic poisoning from a PA is a megalocytosis of the liver in which the liver becomes composed of a small number of giant cells. The lesions appear as a result of prolonged anti-mitotic effect with an increased rate of cell death which in turn provides a stimulus for growth of liver in bulk but not in cell number (Clark, 1976).

Culvenor *et al.*, (1962) suggested that hepatoxicity is due to the presence of a double bond in the chemical structure of these alkaloids, and that the principle reaction is through an alkyl-oxygen fission of an ester-linkage. Mattocks (1968) supported this theory by suggesting that the active forms of *Senecio* alkaloids are the pyrroles produced by mixed function oxidases. This means that the alkaloids are transformed in the liver by ring dehydrogenases to form soluble pyrroles which are possibly adsorbed to local macromolecular sites in the liver thus exerting cytotoxicity and other biological effects.

Cross-linking of DNA by dehydropyrrolizidines was demonstrated *in vitro* by White and Mattocks (1972). Black and Jago (1970) had shown a similar effect using

dehydroheliotrine, a metabolite of heliotrine, in the liver parenchyma cells resulting in total mitotic inhibition. This was due to interaction of dehydro-heliotrine with the DNA of liver cells.

Monocrotaline and heliotrine were found to exhibit mutagenic activity in repair deficient strains of *E. coli* WP2 *uvr*A in the presence of S9 (Green and Muriel, 1975). Heliotrine and lasiocarpine are known to be mutagenic in TA100 with and without metabolic activation (Yamanaka *et al.*, 1979) and in *Aspergillus nidulans* methionine-biotin deficient strain (Alderson and Clark, 1966). They have also been shown to be mutagenic in *Drosophila* (Clark, 1959, 1963, 1966 and 1982). They also cause chro-mosomal aberrations in plant cells, *Drosophila* and human peripheral lymphocytes (Avanzi, 1961; Brink, 1969; Bick and Brown, 1972). Lasiocarpine and heliotrine have also been shown to have a strong clastogenic effect in foetal liver erythrocytes as demonstrated by the use of placental micronucleus test (Stoyel and Clark, 1974).

Lasiocarpine, echimidine and symphylline occur in the russian comfrey, *Symphytum officinale* (Hirono *et al.*, 1978), which is used as herbal tea and as a constituent of salads. These alkaloids were shown to be mutagenic in *Drosophila* (Clark, 1960). However, chopped dry leaves from the russian comfrey failed to show any evidence of mutagenicity either when cooked in the food medium

on which *Drosophoila* larvae are reared on or when used to make an infusion to feed adult males (Clark, 1982). This would mean that comfrey tea made from this plant may not cause genetic damage but may not be ruled out when it is used in fresh salads.

Flavonoids

Flavonoids are another class of plant secondary products known to have mutagenic activity. They occur widely in plants used as human foods and in drug preparations (Brown, 1980). The structural requirements for flavone or flavanol to show mutagenic activity in the Ames test has been reviewed by MacGregor and Jurd (1978) and Brown (1980). All mutagenic flavonoids have a hydroxyl group on carbon position 5 and 3 in their structures. Hydrolysis of flavonoids by glucosidases liberates mutagenic flavones and flavanols (Brown, 1980).

Quercetin and kaempferol are mutagenic in the *Salmonella* strains TA98 and TA1537 (Hardigee and Epler, 1978). Quercetin was more active of the two and even more so after metabolic activation. Its mutagenic activity has also been reported by Bjeldanes and Chang (1977). Quercetin caused single-strand DNA breaks in the mouse L5178Y lymphoma system (Meltz and MacGregor 1981). Other flavonols, morin, fezetin, quercetin and rutin are also mutagenic in microbial test

systems with metabolic activation (Brown, 1980), but quercetin is thought to be the most mutagenic of all flavanols detected in the Ames test. Sieno *et al.*, (1978) showed that the mutagenic compound in the spice, sumac, produced from the seeds of *Rhus*, is quercetin. Uyeta *et al.*, (1981) also showed that mutagenic activity of green tea in the *Salmonella* strains TA98 and TA100 was mainly due to quercetin, kaempferol and myricetin and that metabolic activation was not required. Clark (1982) showed that the flavanols, rutin and aglycone, do not exhibit significant mutagenic activity in *Drosophila*.

Quinones

Quinones also occur naturally in plants used as human food (Brown, 1980; Ames, 1983). Quinones serve as substrates for a wide variety of flavoenzymes, including NADPH-cytochrome P450 reductase, NAD(P)H-quinone oxidoreductase and can undergo direct two electron reduction to semiquinone radical (Lind, 1982). Chesis *et al.*, (1984) demonstrated mutagenicity of 4 quinones namely menadione, benzo(a)pyrene 3-6-quinone, 9,10-phenatrenequinone and danthron in the *S. typhimurium* strain TA104. Tikkanen *et al.*, (1983) have also demonstrated mutagenicity of naturally ocurring quinones in *S. typhimurium* strain TA2637 after metabolic activation. Ashwood

et al. (1982) showed that dictamine, a naturally occuring furoquinone produces frameshift mutations in the dark and does not form DNA interstrand cross-links in *E. coli* cells in the near ultra-violet light (300-380nm). Brown (1980) has reviewed other quinones mutagenic in the *S. typhimurium* test system. Clark (1982) was able to show mutagenic activity of juglone 5-hydroxyl-1,4-naphthoquinone in adult male *Drosophila*. Mutagenic activity was mainly in the first brood.

Cycasin

Cycasin occurs in the seeds, roots and leaves of cycad plants (Family Cycadaceae), which are found in tropical and subtropical regions of the world. The making of starch from cycad nuts is both a cottage and commercial activity in these areas. Cycad starch from nuts of *Cycas circinalis* and *Cycas revoluta* is used in Mariana islands, Ryuku island (Japan), Indo-China, India and Africa (IARC monograph, 1976). In these areas the seeds are also prepared for use both as external and internal medicine (Whiting, 1963). Frequent occurence of toxicosis and neurological disease in livestock and humans in these regions have prompted an intensive investigation of the constituents of cycads.

Mugera and Nderito (1968) observed induction of

tumours in liver, kidney and lungs of rats after chronic ingestion of starchy kernels of a cycad *Encephalatos hilderbrantii*. Smith (1966) showed that cycasin extracted from seeds of *Cycas circinalis* is toxic after being given enterically to guinea pigs but was not toxic when para-enteral injections were given. Its metabolite, methyl-azoxymethanol-B-D-glucoside (MAM) was found to be carcinogenic, independent of the route of administration. MAM had earliar been shown to produce hepatomas in rats (Matsumoto and Strong, 1963). Shank and Magee (1967) showed that cycasin inhibited leucine incorporation into liver proteins but not in the kidney and spleen 5 hr after administration, in experimental animals. They also showed that cycasin and dimethylnitrosamine are biochemically metabolized to the same compound, possibly diazomethane, and that different tissues differ in their capacity to metabolize the two carcinogens. Cycasin and MAM cross the placental barrier in rats and hamsters (Spatz *et al.*, 1967; Spartz and Laquer, 1968) producing malformations of the nervous system of foetuses born of females injected with 20-30 mg/kg body weight of MAM on the 8th day of pregnancy.

MAM prepared by treating cycasin with B-glucosidase was found to induce sex linked recessive lethals when fed to *D. melanogaster* males (Teas and Dyson, 1967). MAM derived from crystalline cycasin induced reverse

mutations in *S. typhimurium*, although the parent compound was inactive (Smith, 1966). Gabridge *et al.*, (1969) showed that intestinal bacteria are involved in the formation of MAM from cycasin. In a host mediated assay, that prior treatment of swiss mice with ampicillin, which reduces the enteric bacterial population, mutagenicity of *S. typhimurium* hisG46 was abolished. Mutagenicity is due to the N-nitroso structure also present in 1-methyl-3-nitro-1 nitrosoguanidine (MNNG) which is a frameshift mutagen in bacteria (Ames, 1971). The n-nitroso structure is reponsible for DNA alkylation resulting in mutagenic effects.

Sinigrin

Sinigrin from cruciferous plants like cabbage and cauliflower is also mutagenic. Allylisothiocyanate, an enzymic hydrolysis product of sinigrin, was found to be mutagenic in *D. melanogaster* (Auerbach and Robson, 1944).

Bracken fern toxin

Bracken fern, *Pteridium aquilinium* is poisonous to livestock, particularly cattle. Two toxicity syndromes result in stock after feeding cattle on fresh or dry bracken. One can be attributed to thiaminase in the plant and is essentially a thiaminase deficiency called B1 avitaminosis which causes chronic neurological symptoms. The second syndrome is similar to those

caused by radiation and radiomimetic chemicals (Evans, 1968).

Carcinogenic substances in bracken have been reported (Evans and Mason, 1965). Evans and Osman (1974) thought that shikimic acid is one of the constituents of bracken but Ishidate and Odashima (1977) were unabe to show any clastogenic effects of shikimic acid in the Chinese hamster ovary cells *in vitro*. Clark (1982) showed that fresh bracken fern extracts are highly mutagenic in *D. melanogaster*, particularly in the first and second broods. Leach *et al.*, (1971) isolated an ethylacetate fraction which causes haemorrhage and denudation of intestinal mucosa of rats after 10 mg i.p. injection but failed to characterize this fraction. A methanol soluble fraction [3] from freeze dried bracken fronds has been shown to be mutagenic in *Salmonella* strains TA98 and TA100 especially in the presence of metabolic activation (van der Hoeven and van Leewen, 1981).

Plant mutagens in crude drugs

Mutagens in crude drugs have also been reported. Shehab (1980) showed that water extract of *Teucrium pilosum*, a plant used against constipation in Quatar, had anti-mitotic effects in roots of *Allium cepa*. Nakamura and Yomoto (1982) showed that juice from ginger, *Zingiber officinale*, a chinese med-

icinal herb, increased mutagenicity of N-methyl-N -nitroguanidine (NTG) and 2-aminofluorene (2-AF) in HS30 cells of *E. coli*. Marimoto *et al.*, (1982) showed mutagenicity of 45 samples of crude drugs using *Bacillus subtilis* rec assay and *Salmonella* microsome assay. Mutagenicity of dictamine and Y-fagarine from *Dictamni radicis* a component of Chinese medicine was reported by Mizuta and Kanamoni (1985). Marimoto *et al.*, (1983) reported mutagenicity of gentisn and isogentisn hydroxanthones in the methanol extract of *Gentinae radix* a plant widely used as a component of bitter drugs in Chinese medicines. These fractions were mutagenic in *Salmonella* TA100 in the presence of metabolic activation. Uwaifo *et al.*, (1979) reported mutagenic activity of a phytochemical compound called chamvaritin isolated from *Uvaria chamnae*, a medicinal herb used as a purgative in Nigeria. Chamvaritin was mutagenic in *Salmonella* strains TA100 and TA98 in the presence of metabolic activation.

1.2.2.3. Plant antimutagens

Antimutagens in plants have also been reported. For example vegetable juice of ginger (Kada *et al.*, 1978) and cabbage (Morita *et al.*, 1978) were found to inhibit mutagenic activity of protein and amino acid

tryptophan pyrolysates that are mutagenic in bacteria (Nagao *et al.*, 1977). Nakamura and Yamamoto (1982) reported that ether soluble fraction isolated from *Z. officinale* was anti-mutagenic. They demonstrated that 200 µg/ml of the gingerol [6] fraction accelerated mutagenic activity of AF2 and NTG in HS30 cells of *E.coli* and that when 0.2 ml of the ether soluble fraction was added to gingerol [6] fraction, mutagenic activity was reduced. Antimutagenic activity has also been reported in other crude drugs used in Chinese medicines such as *Isodonis herba* and *Cinnamoni cortex* (Kakinuma *et al.*, 1984a and b).

The plant flavonoids, rutin, myricetin and quercetin were shown to inhibit genotoxic effects of cooked food pyrrolysates but polyphenolics, caffeic acid, chlorogenic acid, ellargenic acid and ferulic acid did not (Alldric *et al.*, 1986). Many plant extracts have been shown to ameliorate a number of diverse genotoxins (Ishii *et al.*, 1984). Wood *et al.*, (1982) also reported anti-mutagenic activity of polyphenolic acid and its metabolites in bacterial assays. Quercetin exhibits comutagenic activity with 2- acetylaminofluorene (Huang *et al.*, (1983) but shows antimutagenic activity with benzo(a)pyrene (Ogawa *et al.*, 1985).

Thus, to evaluate the safety of administering crude drugs to patients over long periods, it is important to

investigate what levels of mutagenic activity is contained in them. Although literature survey has revealed that plants contain mutagenic compounds, this is of little value for the evaluation of possible genetic effects of medicinal plants used in Kenya, since: (i) local herbalists prescribe crude drug preparations whose chemical constituents are not known; and (ii) where phytochemists are engaged in the chemical analysis of local drug plants, the procedure is rather slow and time consuming so that little progress has been made in this line of research in Kenya.

1.3. Objective of this study

Plants are generally known to act as a source of potential genotoxic ingredients. The aim of the current study is to assess the potential genotoxic effects of some medicinal plants commonly used in Kenya using standard tester systems. The results obtained will indicate the suitability of the continued or projected use of the plants investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1. Reagents

L-histidine.HCl (monohydrate) was obtained from BDH Chemical Company (Poole, England). D-biotin, nicotinamide adenine dinucleotide phosphate (NADP, sodium salt), glucose-6-phosphate (G-6-P) and agar were from Sigma Chemical Company (St. Louis, U.S.A.). Ampicillin trihydrate was a gift of Carol Jansen of Bristol Labs (U.S.A.). Nutrient broth and dimethyl sulfoxide (DMSO) were from E. Merck (W. Germany). ICR-191 was obtained from Polyscience Inc. (U.S.A.) while 2-amino- fluorene (2-AF) was obtained from Aldrich (Milwaukee, U.S.A.). Aroclor 1254 was purchased from Analabs, Inc. (New Haven, Conn.). Tetracycline was locally purchased at Chemitex Limited (Nairobi, Kenya) while skim milk was obtained from Oxoid Chemical Company (London, England).

2.2. Extracts from plants

The list of plants used in the current study and their local names are provided in Table 1. The methanol extracts of *Maesa lanceolata (M. lanceolata)* and *Mrysine africana (M. africana)* were obtained according to the

Table 1. Plants used in the present study

Scientific name	Common name-language	Plant family	Medicinal use	Source of plant material
Aloe graminicola (*A. graminicola*)	kiluma-kikamba	Liliaceae	Sap from plant is used to cure malaria, headache, pneumonia, conjuctivities, chest pains, improve appetite and also as a general disinfectant (Kokwaro, 1976).	Whole plant was obtained from Naivasha.
Annona senegalensis (*A. senegalensis*)	oholo-dholuo	Annonaceae	A decoction of the roots is used as a remedy for chest pains and the gum is applied on wounds to seal them (Watt, 1962; Kokwaro, 1976).	Methanol extract of the roots was obtained from the International Centre of Insect Physiology and Ecology (I.C.I.P.E.).
Centella asiatica (*C. asiatica*)	-	Umbelliferae	The plant has been reported to cure cases of syphilis and scrolofulous conditions (Watt, 1962; Kokwaro, 1976).	Methanol extract of the root was obtained from I.C.I.P.E.
Maesa lanceolata (*M. lanceolata*)	kalatera-dholuo	Mrysinaceae	Fruits are used as a purgative, they are also used in the treatment of sore throat and as an antihelminth (Watt, 1962; Kokwaro, 1976).	Fruits were obtained from Kakamega forest.
Myrsine africana (*M. africana*)	mugaita-kikuyu	Myrsinaceae	Fruits are used to cure chest pains, stiff joints and also as an anti-helminth (Watt, 1962; Kokwaro, 1976)	Fruits were obtained from Naivasha.
Myrica salicifolia (*M. salicifolia*)	kitaloswa-kimurakwet	Myricaceae	Roots are used to ease stomach pains and indigestion (Kokwaro, 1976)	The methanol extract of roots was obtained from the Kenya Medical Research Institute (K.E.M.R.I.)

method described by Harbone (1973). Sun dried seeds were thoroughly crushed using a mortar and pestle and 100 g of the fine powder was soaked in 200 ml of methanol for 2 weeks. The extract so obtained was filtered and the filtrate was evaporated under vacuum. A brown gummy extract of about 5 g for each plant extract was obtained. This extraction was carried out in the Department of Phytochemistry of the International centre of Insect Physiology and Ecology (ICIPE) under instructions of Dr. I. Jodiko.

The sap of *Aloe graminicola (A. graminicola)* was obtained by squeezing it out from leaves of the plant. All the other plant extracts were obtained ready from source shown in Table 1.

2.3. Bacterial tester strains

The *Salmonella typhimurium (S. typhimurium)* tester strains were generously provided by Professor B.N. Ames of the University of California, Berkely. They consisted of TA1535 and the standard set of tester strains designated; TA97a, TA98, TA100 and TA102 (Maron and Ames, 1983). Also included was TA104 which efficiently detects oxidative and natural carbonyl mutagens (Marnett *et al.*, 1985). A summary of the properties possessed by these strains is outlined in Table 2.

Table 2. The *Salmonella* tester strains used in the present study

Strain	Genotypes				Type of mutation detected	Reference
	his mutation	LPS	Repair	R-factor		
TA97a	his01242	*rfa*	*uvrB*	+	Frameshift	Ames, personal communcation.
TA98	his03052	*rfa*	*uvrB*	+	Frameshift	Yourno (1974); McCann *et al.*, (1975b).
TA100	hisG46	*rfa*	*uvrB*	+	Base-pair substitution	Ames (1971); McCann *et al.*,(1975b).
TA102*	hisG428	*rfa*	+	+	Base-pair substitution	Levin *et al.*, (1982b) Levin *et al.*, (1984).
TA104	hisG428	*rfa*	*uvrB*	+	Base-pair substitution	Levin *et al.*, 1982b.
TA1535	his 946	*rfa*	*uvrB*	-	Base-pair substitution	McCann *et al.*, 1975b.

rfa = LPS = Partial loss of polysaccharide barrier.
uvrB = *uvrB* repair damage.
R-factor = Presence of pKM101 plasmid.
* = PQAl plasmid in TA102.
+ = R-factor present
- = R-factor absent

2.4. *Vicia faba (V. faba)* seeds

The seeds (lot number 3225) were bought from Simlaw Seeds Company (Nairobi).

2.5. Rats

55 days old, male Wistar rats, were obtained from the Department of Zoology, of the University of Nairobi.

2.6. The *Salmonella/* mutagenicity test

The revised methods of Maron and Ames (1983) were used in this study. All glassware, media and reagents were sterilized before each experiment.

2.6.1. Preparation of stock solutions and media

These were prepared according to Maron and Ames (1983). Phosphate buffer saline (PBS) was prepared according to Wasley and May (1970).

2.6.2. Reisolation of tester strains

The tester strain cultures were re-isolated according to the instructions accompanying the cultures from Professor B.N. Ames.

2.6.3. Maintenance of cultures

Lyophilized preparations

Lyophilized cultures were made from skim milk suspension according to the method of Maron and Ames (1983). The lyophilized cultures were kept at 4°C. When cultures are preserved in this form, they have been found to stay for 3 years or more without loss of viability or genetic markers (Maron and Ames, 1983).

Master plates

These were also prepared according to Maron and Ames (1983). The purpose of these plates was to serve as the routine source of bacteria for inoculating overnight cultures. These plates were stored at 4°C. Master plates were never kept for more than two months because spontaneous revertants counts have been found to increase when master plates are too old (Maron and Ames, 1983). Master plates containing TA102 were discarded after two weeks and fresh ones made from lyophilized cultures. This is because tetracycline added into the media to retain the PAQ1 plasmid increases the spontaneous reversion frequency of TA102 (Levin *et al.*, 1982b).

Overnight cultures

These cultures were grown according to Maron and Ames (1983) but for 16 hours at 37^{0}C and without shaking. However, the cultures were shaken just before use. This culture contained between 1-2 x 10^9 live cells/ml as determined by the Miles and Misra drop plate method (Somasegaran *et al.*, 1979).

2.6.4. Confirming genotypes

The genotypes of the tester strains (Table 2) were always confirmed immediately before and after preparing lyophilized cultures; and when a new set of lyophilized cultures was harvested. The various genotypes were confirmed as follows:

Histidine requirement

The "his-" character of the tester strains was confirmed by demonstrating histidine requirement for growth on a histidine-biotin plate. A sterile cotton swab was dipped into overnight culture (16 hr) and excess squeezed out. The cotton swab was then swept across a histidine-biotin plate. The plates were then incubated overnight at 37^{0}C. A control was set in an identical way but the plates contained biotin but no histidine. Growth of all tester strains in the histidine-biotin plate but no growth in the control

plate confirmed histidine requirement.

rfa mutation

Strains with deep rough (*rfa*) mutation were tested for crystal violet sensitivity (Maron and Ames, 1983). A 2 ml sample of molten top agar was dispensed into a culture tube held at 45°C on a heating block and 0.1 ml of fresh overnight culture was added. The mixture was then vortexed for about 3 seconds and then poured on a nutrient agar plate. The plate was tilted and rotated to distribute the agar evenly. The plate was then placed on a level surface to get firm and thereafter a sterile disc (5 mm in diameter) containing 10 µl of 1 µg/ml crystal violet was placed at the centre of the plate using sterile forceps. The plate was then incubated overnight at 37°C. Presence of a ring of growth inhibition around the filter disc indicated presence of *rfa* mutation.

uvrB mutation

The *uvrB* mutation was confirmed by demonstrating UV sensitivity in strains that contain this mutation (Maron and Ames, 1983). Using a sterile cotton swab, the tester strains were streaked across a nutrient agar plate. A piece of aluminium foil was placed on one half of the uncovered petriplate. The plate was then irradiated

with a UV-lamp 15-W held at a distance of 33 cm above the bench. All the tester strains were tested on the same plate. Growth of the bacteria only on the unirradiated side confirmed *uvr*B mutation. TA102 grew on both the irradiated and nonirradiated sides. This is because its UV-repair system is intact (Levin *et al.*, 1982b).

R-factor

The R-factor plasmid is unstable and can be lost from the bacteria (McCann *et al.*, 1975b). Its presence was confirmed as follows: using a sterile cotton swab, the strains were streaked across an ampicillin plate. A non- R factor strain TA1535 was streaked together with the R-factor strains. Growth of all the tester strains and lack of growth for TA1535 confirmed presence of the plasmid.

PAQ1 plasmid

This was tested by streaking TA102 on an ampicillin-tetracycline plate. A control was set by streaking an R-factor strain TA100 together with TA102. Growth of TA102 and not TA100 confirmed presence of the PAQ1 plasmid.

2.6.5. Metabolic activation

Induction of liver enzymes

The method of Maron and Ames (1983) was used with slight modification. Two 55 day old male Wistar rats weighing 180 and 160 g, respectively, were each given a single i.p. injection of 500 mg/kg body weight of aroclor 1254 diluted in corn oil. The rats were given laboratory feed and water for 5 days but 12 hours before sacrifice, the food and not water was removed.

Preparation of liver homogenate (S9)

The preparation of S9 was done according to the procedure of Maron and Ames (1983). All steps were carried out at 0-4°C using sterile chilled solutions and glassware.

Freshly excised livers were placed in beakers containing 15 ml of 0.15 M KCl and washed several times to ensure a sterile preparation and to remove haemoglobin which can inhibit the activity of cytochrome P450 enzymes.

The washed livers were then quickly minced with a pair of sterile scissors and ground thoroughly using a sterile mortar and pestle. A sample of 45 ml of KCl was added in small portions while constantly homogenizing the preparation with the pestle. The homogenate was then centrifuged for 10 minutes at 9,000g in a refrigerated

centrifuge. The supernatant was decanted and retained.

The freshly prepared S9 was distributed into small nunc tubes (upto 1.8 ml). The tubes were stored immediately in a liquid nitrogen tank. The remaining S9 fraction was placed in an ice bath and tested for sterility by plating a 0.1ml sample on a histidine biotin plate and incubating it at 37°C for 48 hours. Lack of growth of contaminating microorganisms confirmed sterility of the S9 preparation.

2.6.6. Preparation of test solution for use in the Ames/*Salmonella* mutagenicity test.

The fresh sap of *A. graminicola* was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 500 µl/ml. Each of the methanol extracts of the plants shown in Table 1 was dissolved in DMSO to make a stock solution of 10 mg/ml. To remove contaminating microorganisms, the solutions were filter sterilized using a 0.15 µm Göttigen membrane filter. A sample of 0.1 ml of each of the sterile solution was plated on a histidine-biotin plate and incubated at 37°C for 48 hours. Lack of growth of contaminating microorganisms confirmed sterility of the stock solution.

The stock solution was then diluted with DMSO before each experiment to obtain the following concentrations: 10, 5, 2.5, 1.25 and 0.625 mg/ml for the

methanol extracts and 500; 250, 125, 62.5 and 31.25 µl/ml for the sap of *A. graminicola*.

2.6.7. Mutagenicity test

The standard plate incorporation test of Maron and Ames (1983) was performed. A 10 ml sample of a sterile solution of 0.5 mM L-histidine.HCl/0.5 mM biotin was added to 100ml molten top agar, thoroughly mixed and 2ml distributed to sterile capped culture tubes held at 45°C on a heating block. To this agar, the following were added in the order given; 0.1 ml of fresh overnight culture, 0.5 ml of 0.2M sterile phosphate buffer (pH 7.4) and 0.1 ml of sterile plant extract.

The mixture was vortexed for about 3 seconds and then poured on minimal glucose agar plates. Triplicate plates were prepared for each dose of plant extract. A negative control using triplicate plates of bacteria and solvent (DMSO) was set. The purpose of these plates was to confirm the spontaneous reversion properties of each strain. Also included in this test was a positive control whereby a single plate of 1.0 µg/plate of ICR-191 was used for all tester strains. Although each strain is diagnosed using specific mutagens (Maron and Ames, 1983), ICR-191 was used because it shows different reversion responses in all the standard tester strains (Maron and Ames, 1983).

An identical experiment was repeated whereby 0.5 ml of S9 mix was also added. The negative control for this experiment included solvent and S9 mix. The positive control for the standard strains (TA97a, TA98, TA100 and TA102) with S9 was the known mutagen, 2-aminofluorene (2-AF). No positive control was available for TA104.

The plates were then incubated at 37^{0}C for 48 hr after which revertant colonies were counted using a GallenKamp Colony Counter. The presence of lawn was first confirmed in all plates before counting was done. The results of the revertant colonies was expressed as mean number of revertant colonies per plate (Table 3a-f).

2.6.8. Analysis of the *Salmonella* mutagenicity test Data

Data was analysed using a MEI mathematical program (Couzen and Butler, 1985) on a BBC microcomputer. One way analysis of variance (ANOVA) was used to determine whether the mean number of revertants scored per plate at different treatment levels with the plant extract were significantly different.

The significance of the F-ratio (Appendix 1a-f) was justified using the mathematical tables of Freund (1972). The degrees of freedom associated with variation due to treatment were read across the top (numerator)

and those associated with variation due to dispersion among plates were read downwards (denominator).

When the observed F value is less than the F expected at 5% level (F, 0.05), there is no real difference among treatments but if it is greater than F required at 5% level, then there is a probability of 5% or less ($P \leq 0.05$) that the observed variation among treatments occurred by chance.

Whenever the F-test was significant, the least significant difference (LSD) method was used to determine which of the means arranged in order of magnitude were significantly different (Appendix, 2a-f). The LSD is calculated as follows;

$$LSD = t\sqrt{\frac{2(MSE)}{r}}$$

where t is a tabulated value chosen for the degrees of freedom for error and at level of significance desired, MSE is the mean square for error, r is the number of variants (replicates in this study) on which the means to be separated are based. Means followed by a common letter are not significantly different (appendix 2a-f).

2.7. Roots of *Vicia faba (V. faba)* for the study of induced chromosomal aberrations.

The method of Kihlman (1971) was used with slight modifications.

2.7.1. Method for growing roots from seeds.

Seeds were soaked in running tap water for 1-2 days and then transferred into a bed of wet vermiculite. They were then allowed to germinate for 3-4 days at 20°C and by the end of this period had grown primary roots (3-6 cm). The tips of these roots were chopped off and the seedlings were replanted in fresh wet vermiculite and grown for a further 4 days. By the end of this period lateral roots of 1-2 cm length had emerged and these were ready for treatment.

2.7.2. Preparation of treatment solutions.

The treatment solutions were prepared fresh, before each experiment. A stock solution of 500mg/ml was prepared in DMSO for the methanol extracts. This was further diluted in distilled water (Ma, 1982) to make treatment solutions of 10, 5, 2.5 and 1.25 mg/ml. A control was prepared containing 2% DMSO i.e. the amount of DMSO in the highest concentration. The pH of the treatment solution was adjusted to 7.0 ± 0.2 using a 0.2 M sodium phosphate buffer, pH 7.4. The fresh sap of *A. graminicola* was diluted to treatment doses of 10, 5, 2.5 and 1.25% using distilled water. A control containing water only was set and pH adjusted as above.

2.7.3. Treatment

The roots of intact seedlings were immersed into beakers containing 25 ml of treatment solution in duplicates. They were then placed at 20°C in a dark cupboard. The beakers were shaken every 15 minutes to allow aeration of the gas phase of the treatment solution. In all the experiments, the duration of treatment was kept constant at 2 hours. After treatment, some of the roots were chopped off and fixed while the rest were recovered in running tap water and in the dark for 20 hours.

2.7.4. Fixation

Both the control and the treated roots were fixed in 1:3 acetic alcohol (glacial acetic acid : methanol) overnight at 4°C. When the roots were not immediately stained, they were stored by washing away the fixative in distilled water and then preserving them in 70% ethanol in the freezer.

2.7.5. Staining.

Prior to staining, the roots were removed from the fixative (or 70% alcohol), washed in distilled water and then hydrolysed in 1N HCl at 60°C for 10 minutes in a water bath. They were then stained according to the conventional Feulgen technique (Sharma and Sharma, 1972).

2.7.6. Scoring of slides

Abnormal metaphases

Abnormal metaphases looked "clumpy" or "condensed" (Fig. 9a). These were scored from 4 slides (2 from each of the duplicate experiment) selected at random. A total of 100 metaphases were scored from the various fields of view selected along a straight line on the slide. The frequency of abnormal metaphases was scored against the normal metaphases. This process of scoring was repeated for all doses of treatment and in both roots fixed immediately after treatment and those allowed to recover for 20 hours. In *M. salicifolia* C-metaphases (Fig. 9c) were scored as abnormal metaphases.

Bridges

Bridges when present occured in anaphase (Fig. 13a & b) or telophase (Fig. 13d). The total number of bridges observed per treatment was scored in 100 anaphases and telophases.

Fragments

These were recorded at metaphase and anaphase (Fig. 14a & c). the procedure adopted to score

fragments was similar to that one used in scoring bridges.

Micronuclei

Micronuclei (MCN; Fig. 16a & b) were only observed in roots treated with fresh sap of *A. graminicola* and recovered in running tap water in the dark for 20 hours. Ten fields of view were scored for MCN in 4 slides (2 slides from each of the duplicate experiments). The number of nuclei possessing MCN were scored against those without MCN. The percentage of cells with MCN was recorded at each dose of treatment.

Enlarged nucleoli

These were a common feature in *V. faba* root tip cells treated with the methanol extract of *M. lanceolata* (Fig. 18). The size of the nucleoli was estimated in 10 nuclei in 2 slides selected at random for each dose of treatment. From each slide, 5 fields of view selected along a straight line were examined for the size of nucleoli. The diameter of 10 nucleoli was measured in 10 fields of view giving a total of 100 cells observed in both slides. The mean diameter of the nucleoli in all doses of treatment was estimated using an occular micrometer. The volume of the nucleoli was calculated as that of a sphere (Flanders and Price, 1970).

2.7.7. Analysis of *V. faba* data.

The various cytogenetic effects observed after treatment of *V. faba* root meristems were analysed for statistical significance using Spearman's rank correlation coefficient method and linear regression (Little and Hills, 1978).

In the first method, the correlation coefficient (r) helped to show how the production of cytogenetic effects (dependent variable) was related to the dose of treatment (independent variable).

In the second method (linear regression), the amount of change associated with a unit change in the independent variable was calculated using the simple linear regression equation: $\hat{Y} = a + bx$.
where "$\hat{Y}$" is the estimated value of Y, "a" is the intercept or point where the line crosses the Y-axis, and "b" is the slope or regression coefficient. The regression coefficient (b) was tested for significance using Anova in an F-test (appendix 3-5). A significant b indicates that the observed cytogenetic effects agree with the expected values and that the plant extract in question enhanced their production.

CHAPTER 3

RESULTS

3.1. Section 1: *Salmonella* assay

The plant extracts evaluated for mutagenicity in the *Salmonella* test are shown in Table 1. Throughout these experiments, the standard plate incorporation assay (Maron and Ames, 1983) was conducted. Each plant extract was tested with and without metabolic activation (S9). Doses used for treatment are as indicated in Chapter 2.

3.1.1. *Aloe graminicola (A. graminicola).*

The results obtained after treatment of *Salmonella* with the fresh sap of *A. graminicola* can be seen in Table 3a. It is clear that this extract induced reverse mutations in all tester strains apart from TA98. The increase in revertants was more so after metabolic activation (+S9) in all tester strains except for TA97a where a reduction was noticed (Fig. 3a). As can be seen, this extract significantly enhanced the reverse mutation frequencies over the DMSO controls ($P < 0.05$) for TA97a, TA100 and TA104 (Appendix 1a). For TA102 significant differences over the controls were only observed in the presence of S9 ($P < 0.001$).

Table 3a. Mutagenicity of the fresh sap of *A. graminicola* in the *Salmonella* tester strains.

Dose ul/plate	Revertants /plate (Mean ± S.D.)[A]									
	TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	89±8	100±19	16±4	25±3	250±15	100±14	250±15	258±20	206±6	350±25
3.125	166±27	95±27	15±2	20±4	108±7	106±9	273±15	458±44	243±37	508±9
6.25	159±1	107±17	16±4	24±2	151±31	135±30	299±15	551±29	221±66	563±27
12.5	166±65	109±16	15±2	22±4	153±30	152±18	267±38	549±28	261±21	667±28
25	200±20	134±19	19±5	20±3	170±2	164±7	287±14	532±14	290±9	837±6
50	280±35	152±13	22±4	19±1	168±21	181±16	309±36	542±17	309±7	978±8
Positive control[C]	1196	1032	47	916	248	688	232	324	-	-

A. Mean ± Standard deviation of counts from triplicates.

B. DMSO control value for the tester strain.

C. ICR - 191 served as positive control in the non activated (-S9) test while 2-AF served as the positive control in the activated (+S9) test.

D. Positive controls were not available for TA104.

Fig. 3

Mutagenic effect of the sap of *A. graminicola* in the *Salmonella* tester strains. Each point represents the mean number of revertants in three plates after deduction of appropriate control values.
(e) -S9 and (f) +S9 show the summary effect of the extract in all strains.

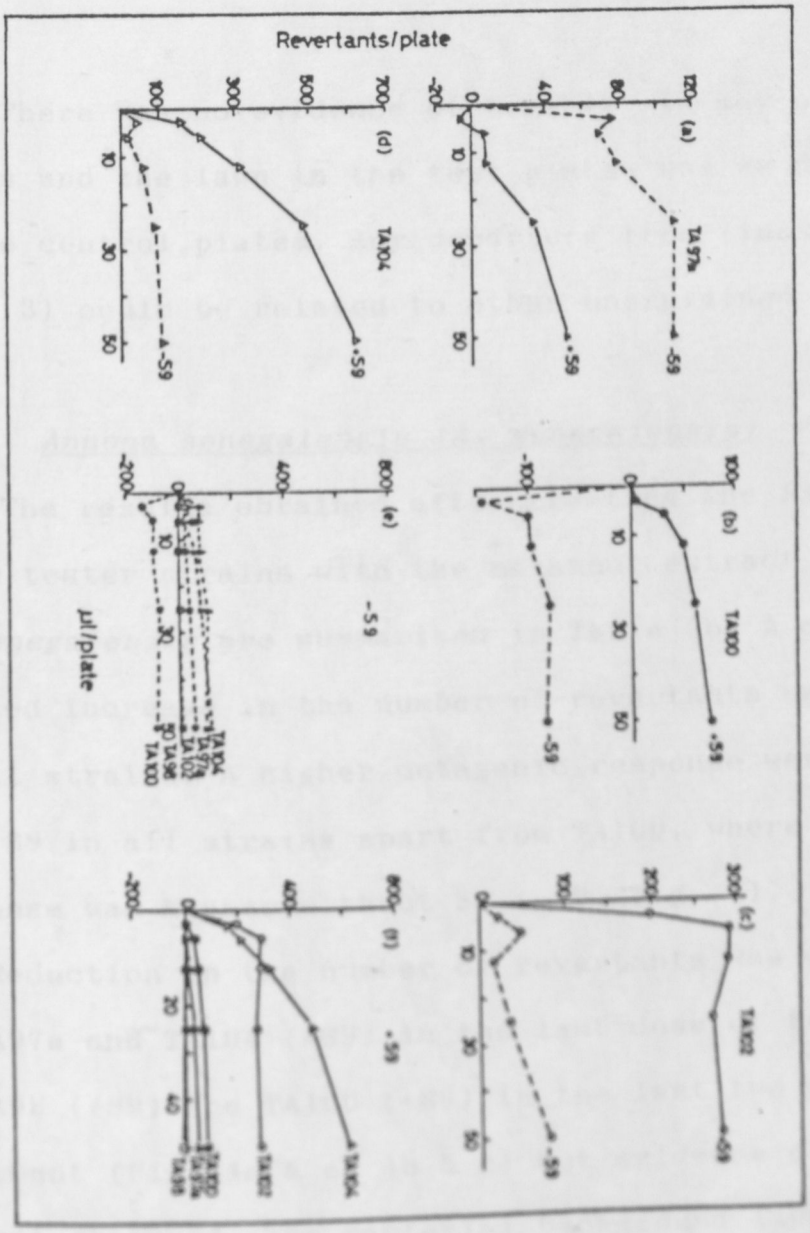

Revertants/plate
(a)
TA97a
-S9
+S9
(b)
TA100
(c)
TA102
(d)
TA104
(e)
-S9
(f)
+S9
µl/plate

Fig. 3

The extract of *A. graminicola* seems to be most mutagenic in TA104 with and without S9 (Fig. 3e & 3f). Only in TA104 (+S9) was the response significantly different (P = 0.05) between all adjacent means (Appendix, 2a).

There was no evidence of toxicity in any of the plates and the lawn in the test plates was as dense as in the control plates. Any departure from linearity (Fig. 3) could be related to other unexplained factors.

3.1.2. *Annona senegalensis (A. senegalensis)*

The results obtained after treating the *Salmonella* tester strains with the methanol extract of *A. senegalensis* are summarised in Table 3b. A dose related increase in the number of revertants was observed in all strains. A higher mutagenic response was observed with S9 in all strains apart from TA100, where the response was higher without S9 (-S9, Fig. 4).

Reduction in the number of revertants was observed in TA97a and TA104 (-S9) in the last dose of treatment and in TA98 (+S9) and TA100 (-S9) in the last two doses of treatment (Fig. 4a & e; 4b & c) but evidence of toxicity was not observed. The bacterial background lawn in all plates was as dense as in the controls. The methanol extract of *A. senegalensis* was most mutagenic in TA100

Table 3b.Mutagenicity of the methanol extract of *A. senegalensis* in the *Salmonella* tester strains.

Dose ul/plate	Revertants /plate (Mean ± S.D)[A] TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	151±43	168±2	28±4	42±6	128±33	166±40	205±6	228±23	341±35	294±11
62.5	351±19	167±2	117±11	142±5	136±11	152±7	312±18	341±22	414±16	342±21
12.5	321±11	244±39	164±30	356±25	257±6	274±11	399±3	451±16	604±7	541±30
250	341±35	322±48	143±23	416±18	644±60	456±23	421±2	480±2	620±1	642±8
500	401±125	356±59	263±32	385±10	529±113	487±18	477±5	515±8	640±1	616±23
1000	317±7	622±18	396±20	316±7	488±23	481±12	513±17	532±2	526±23	843±31
Positive control[C]	1460	1004	63	2010	251	1344	232	456	-	-

A. Mean ± Standard deviation of counts from triplicates.

B. DMSO control value for the tester strain.

C. ICR - 191 served as positive control in the non-activated (-S9) test while 2 -AF served as positive control in the activated test (+S9).

D. Positive controls not available for TA104.

Fig. 4

Mutagenic effect of the methanol extract of *A. senegalensis* in the *Salmonella* tester strains.
Each point represents the mean number of revertants in three plates after deduction of appropriate control values.
(f) -S9 and (g) +S9 show the summary effect of the extract in all srains.

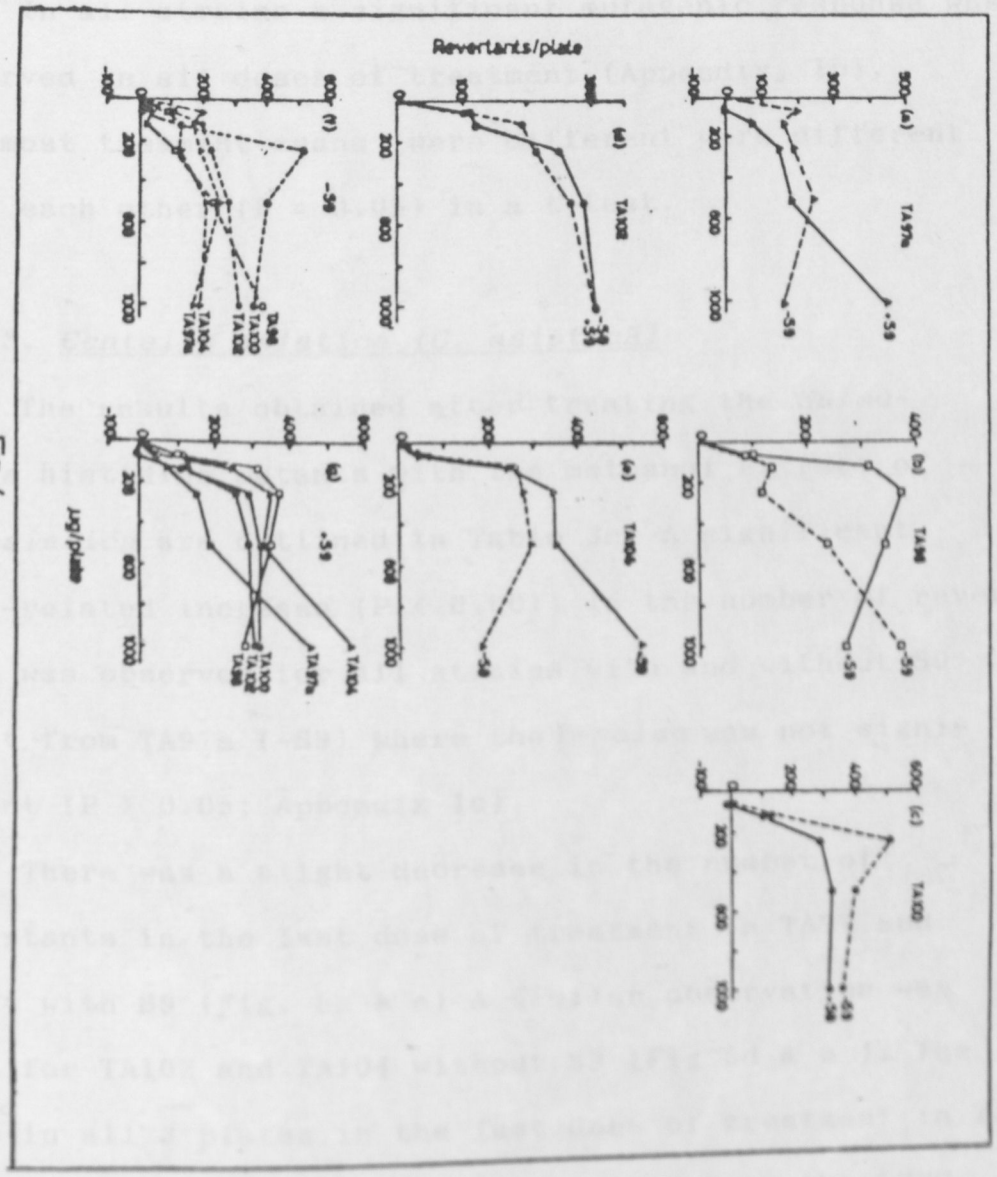

Revertants/plate
μg/plate
TA97a
TA98
TA100
TA102
TA104
-S9
+S9

Fig. 4.

(-S9) and TA104 (+S9) as compared to the other tester strains (Fig. 4f & g).

In all strains a significant mutagenic response was observed in all doses of treatment (Appendix, 1b), and most treament means were different were different from each other (P = 0.05) in a t-test.

3.1.3. *Centella asiatica (C. asiatica)*

The results obtained after treating the *Salmonella* histidine mutants with the methanol extract of *C. asiatica* are outlined in Table 3c. A significant dose-related increase ($P < 0.001$) in the number of revertants was observed for all strains with and without S9 apart from TA97a (-S9) where the F-value was not significant ($P > 0.05$; Appendix 1c).

There was a slight decrease in the number of revertants in the last dose of treatment in TA97 and TA104 with S9 (Fig. 5a & e) A similar observation was made for TA102 and TA104 without S9 (Fig 5d & e). The lawn in all 3 plates in the last dose of treatment in TA97a and TA104 (+ S9) was less dense than that in the DMSO control, but the colony size was the same in both. Thus, the extract was probably not toxic.

3.1.4. *Maesa lanceolata (M. lanceolata)*

In Table 3d the data obtained after treating

Table 3c. Mutagenicity of the methanol extract of *C. asiatica* in the *Salmonella* tester strains.

Dose ul/plate	Revertants /plate (Mean ± S.D)[A]									
	TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	142±18	163±10	30±2	29±3	152±18	121±13	243±34	329±33	341±45	382±18
62.5	166±10	245±8	107±3	168±12	225±20	237±13	331±14	396±29	380±6	405±68
125	167±5	227±6	130±16	198±11	250±31	311±6	402±14	634±42	364±26	564±74
250	137±11	249±17	181±12	216±7	315±7	405±9	454±20	825±34	467±36	472±14
500	157±30	227±6	245±11	302±22	324±11	433±18	476±11	810±22	546±49	616±20
1000	133±8	216±10	255±8	325±10	313±8	499±18	506±10	734±30	526±14	529±35
Positive control[C]	2668	898	68	1412	148	636	148	273	-	-

A. Mean ± standard deviation of counts from triplicates.

B. DMSO control value for the tester strain.

C. ICR - 191 served as positive control in the non-activated (-S9) test while 2 -AF served as positive control in the activated (+S9) test.

D. Positive controls not available for TA104.

Fig. 5

Mutagenic effect of the methanol extract of *C. asiatica* in the *Salmonella* tester strains. Each point represents the mean number of revertants in three plates after deduction of appropriate control values. (f) -S9 and (g) +S9 show the summary effect of the extract in all strains

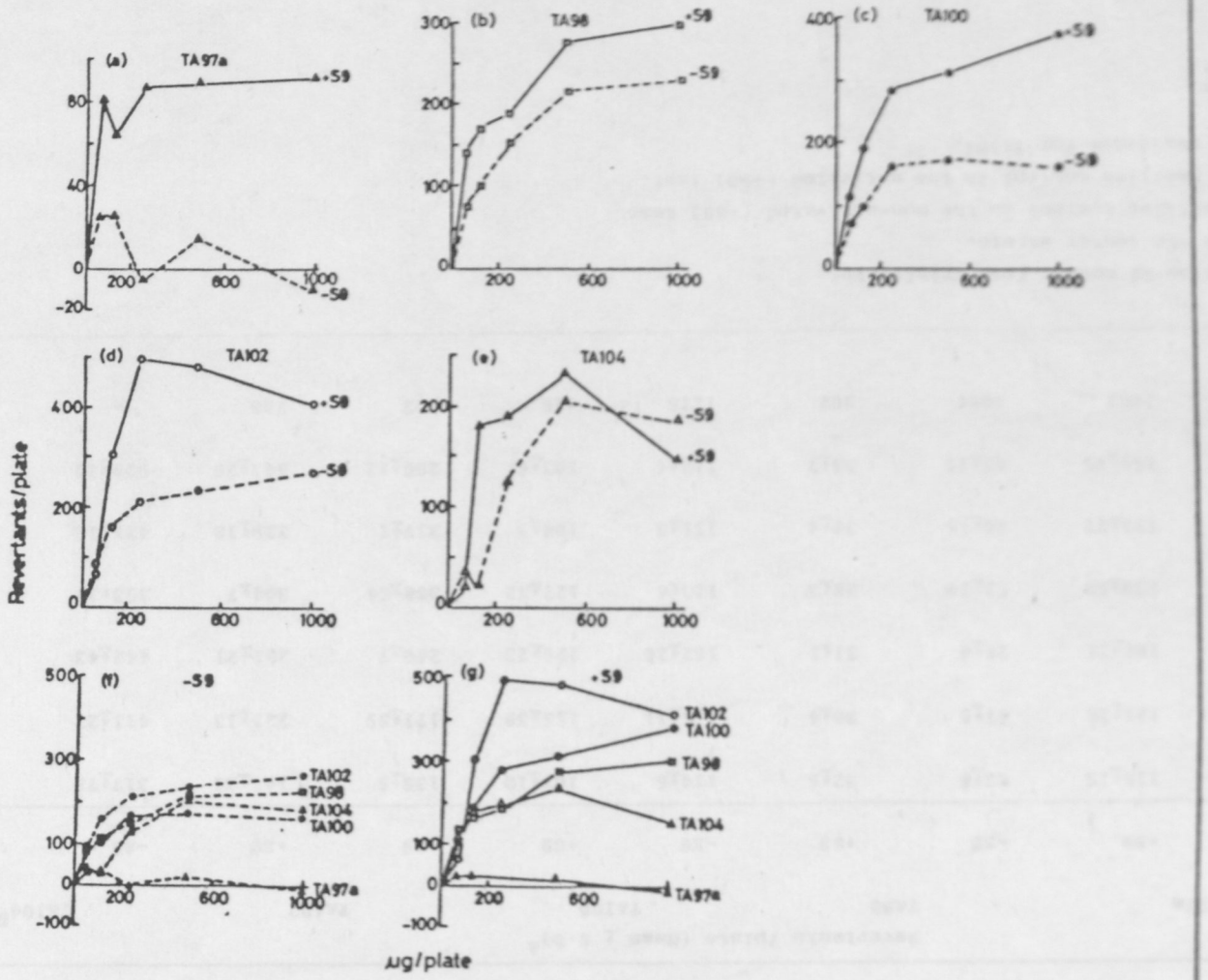

(a) TA97a
(b) TA98
(c) TA100
(d) TA102
(e) TA104
(f) -S9
(g) +S9
+S9
-S9
Revertants/plate
μg/plate
TA102
TA98
TA104
TA100
TA97a

Fig. 5

Table 3d. Mutagenicity of the methanol extract of *M. lanceolata* in the *Salmonella* tester strains.

Dose ul/plate	Revertants /plate (Mean ± S.D)[A]									
	TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	131±50	115±25	42±6	32±5	114±6	107±10	139±5	147±34	317±79	275±1
62.5	209±17	129±20	47±8	30±4	135±11	155±39	171±59	221±13	411±22	365±34
125	186±21	186±21	29±4	31±2	162±26	125±23	240±7	288±21	442±43	437±9
250	216±16	230±50	53±14	38±3	113±6	127±42	308±54	304±7	353+34	493±9
500	179±16	173±27	40±15	39±4	111±8	104±3	337±1	339±18	421±15	489±17
1000	178±8	158±45	53±11	38±7	112+4	103±6	290±12	543±20	529±36	551±39
Positive control[C]	1136	1062	1684	102	1216	216	302	236	-	-

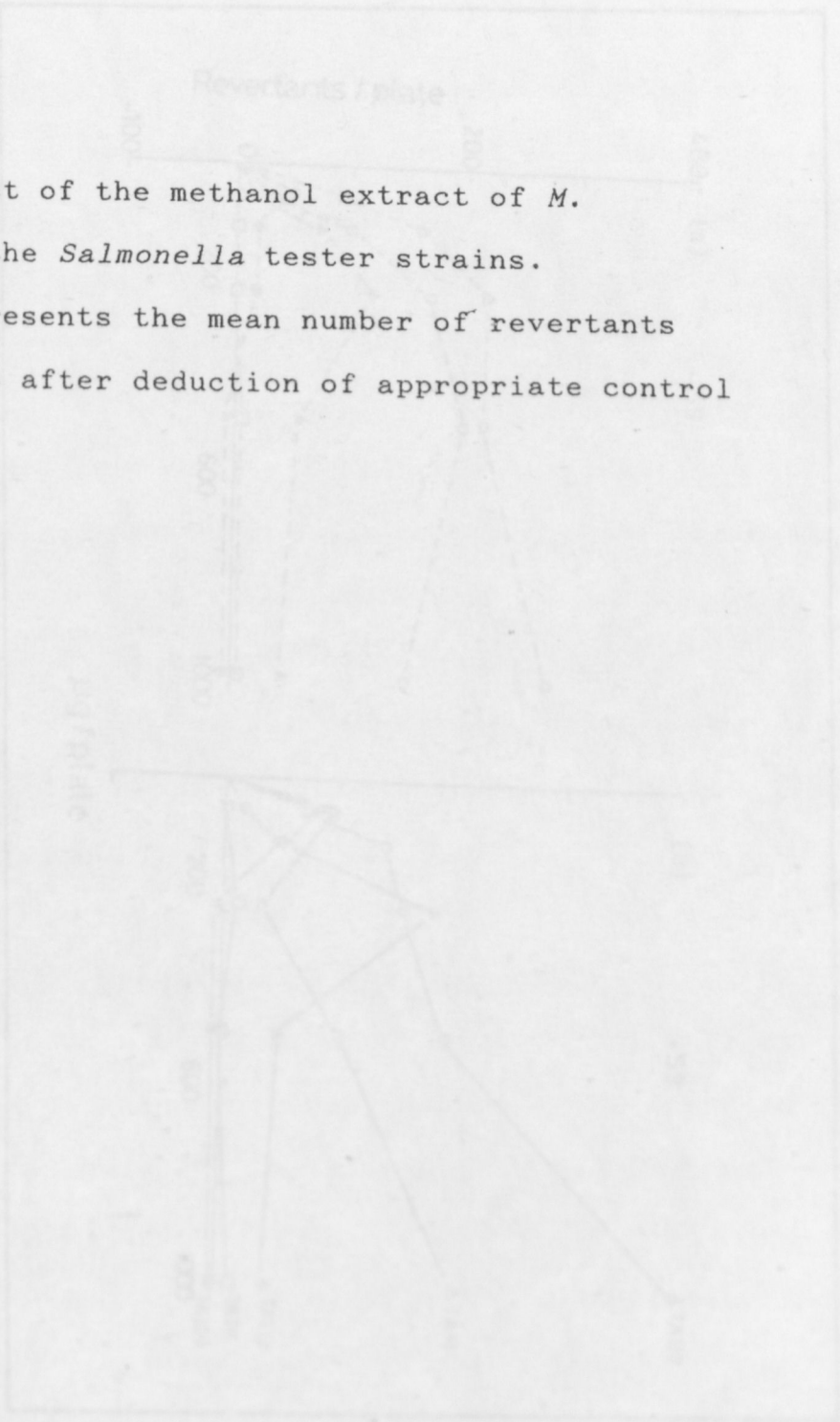
A. Mean ± Standard deviation of counts from triplicates.

B. DMSO control value for the tester strain.

C. ICR - 191 served as positive control in the non-activated (-S9) test while 2 -AF served as positive control in the activated (+S9) test.

D. Positive controls not available for TA104.

Fig. 6



Mutagenic effect of the methanol extract of *M. lanceolata* in the *Salmonella* tester strains.
Each point represents the mean number of revertants in three plates after deduction of appropriate control values.

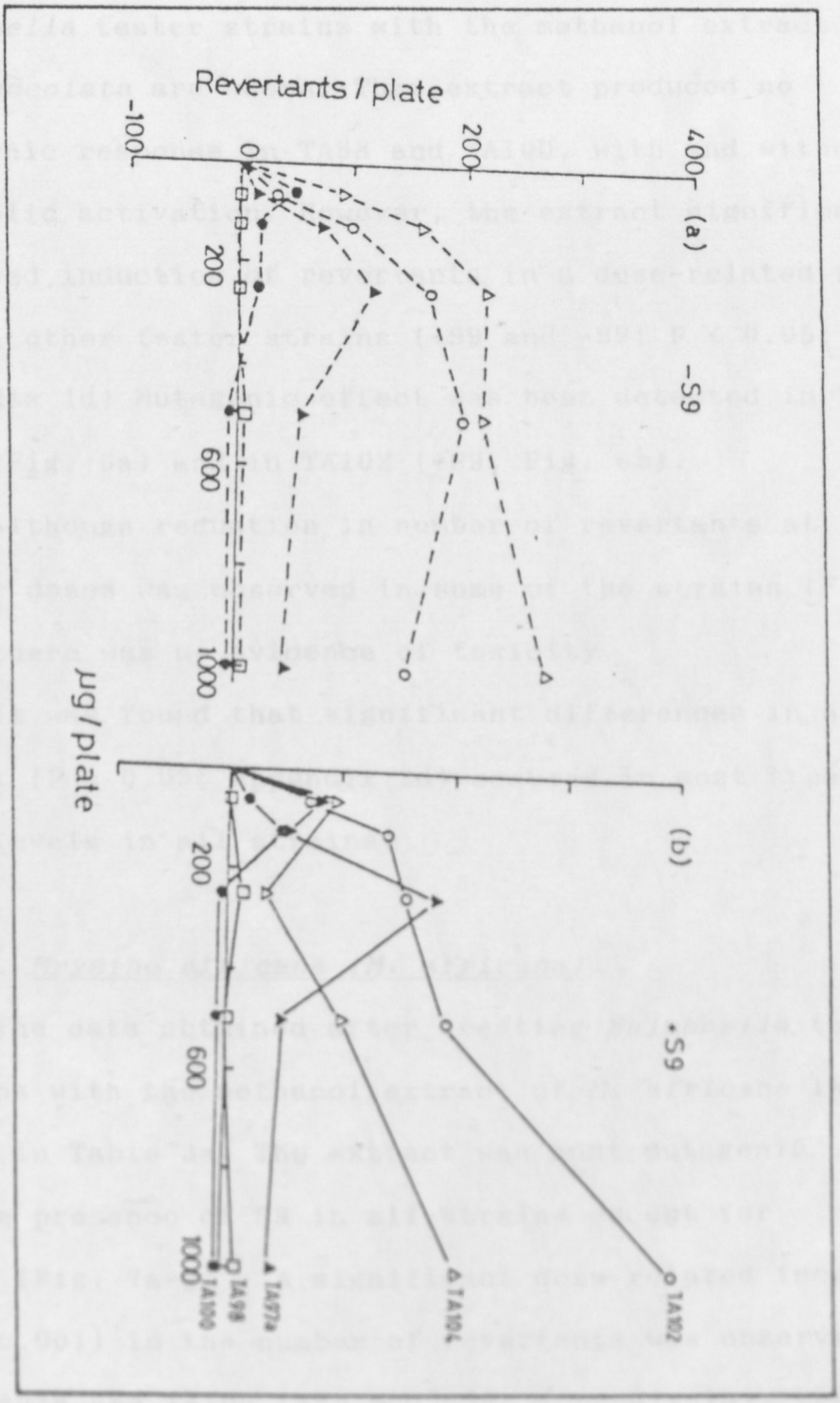

Revertants / plate
-100
0
200
400
(a)
-S9
200
600
1000
µg/plate
(b)
+S9
200
600
1000
TA98
TA100
TA97a
TA104
TA102

Fig. 6

Salmonella tester strains with the methanol extract of *M. lanceolata* are shown. This extract produced no mutagenic response in TA98 and TA100, with and without metabolic activation. However, the extract significantly enhanced induction of revertants in a dose-related fashion in all other tester strains (+S9 and -S9; $P < 0.05$; Appendix 1d) Mutagenic effect was best detected in TA104 (-S9, Fig. 6a) and in TA102 (+S9, Fig. 6b).

Although reduction in number of revertants at higher doses was observed in some of the strains (Fig. 6a & b) there was no evidence of toxicity.

It was found that significant differences in a t-test ($P = 0.05$; Appendix 2d) occured in most treatment levels in all strains.

3.1.5. *Mrysine africana (M. africana)*

The data obtained after treating *Salmonella* tester strains with the methanol extract of *M. africana* is shown in Table 3e. The extract was most mutagenic in the presence of S9 in all strains except for TA102 (Fig. 7a-g) . A significant dose-related increase ($P < 0.001$) in the number of revertants was observed for TA97a and TA104 (+S9 and -S9; Appendix 1e). For TA98 and TA100 there was a significant dose-related increase ($P < 0.05$) in the number of revertants only with S9 (Appendix 1e). In TA102 a significant increase in the number of revertants was only observed without S9 ($P < 0.05$)

Table 3e. Mutagenicity of the methanol extract of *M. africana* in the *Salmonella* tester strains.

Dose (ul/plate)	Revertants /plates (Mean± S.D)[A]									
	TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	91±16	135±5	32±4	37±5	108±2	103±26	321±8	329±7	310±7	321±1
62.5	93±12	153±31	23±4	95±24	107±6	159±11	323±51	317±5	328±14	350±13
125	128±16	147±25	22±8	176±97	117±5	132±9	301±3	318±4	407±27	504±7
250	151±25	213±31	23±1	253±112	106±5	122±18	350±21	334±14	367±12	595±4
500	151±21	321±40	24±4	253±18	106±4	123±6	338±41	322±2	366±12	769±15
1000	109±13	422±24	27±6	192±73	106±4	115±8	384±43	365±28	323±30	632±6
Positive control[C]	820	1208	54	1148	139	1080	339	288	-	-

A. Mean ± Standard deviation of counts from triplicates.

B. DMSO control value for the tester strain.

C. ICR - 191 served as positive control in the non-activated (-S9) test while 2 -AF served as positive control in the activated (+S9) test.

D. Positive controls not available for TA104.

Fig. 7

Mutagenic effect of the methanol extract of *M. africana* in the *Salmonella* tester strains.
Each point represents the mean number of revertants in three plates after deduction of appropriate control values.
(e) -S9 and (g) +S9 show the summary effect of the extract in all strains.

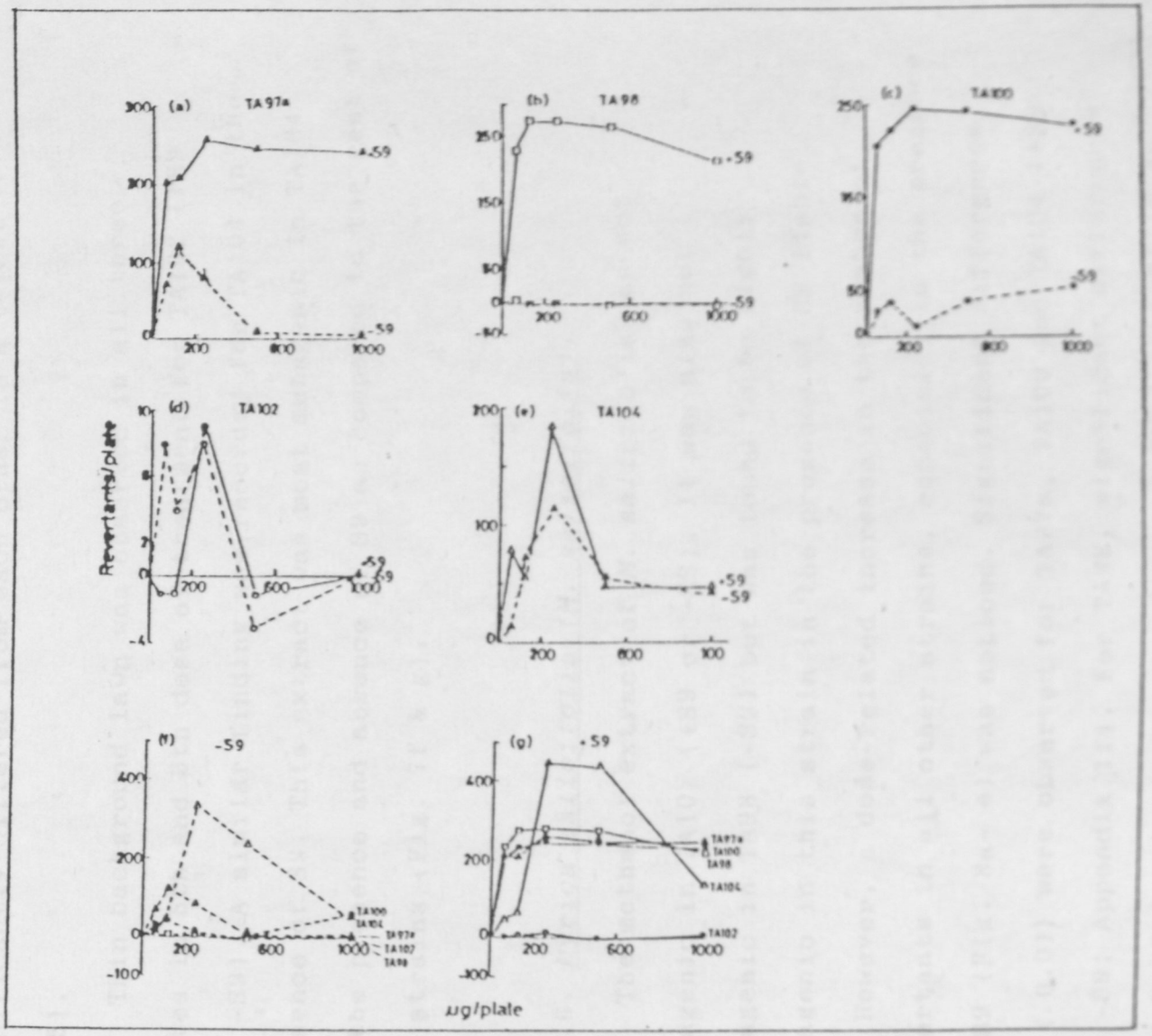

(a) TA 97a
(b) TA 98
(c) TA 100
(d) TA 102
(e) TA 104
(f) -S9
(g) +S9
Revertants/plate
μg/plate
+S9
-S9
TA 100
TA 104
TA 97a
TA 102
TA 98

Fig. 7

but not without it (Appendix 1e).

It can be seen in Appendix 2e that only in TA104 (+S9) that the response between each pair of adjacent means significantly differed from each other in a t-test (P = 0.05).

Thin background lawn was observed in all three plates in 5th and 6th dose of treatment for TA102 (+S9 and -S9). A similar finding was recorded for TA104 in the presence of S9. This extract was most mutagenic in TA104 in the presence and absence of S9 as compared to the rest of the strains (Fig. 7f & g).

3.1.6. *Myrica salicifolia (M. salicifolia).*

The methanol extract of *M. salicifolia* was not mutagenic in TA102 (+S9 or -S9). It was also not mutagenic in TA98 (-S9) but was found to be highly mutagenic in this strain in the presence of S9 (Table 3f) However, a dose-related increase in the number of revertants in all other strains, especially in the presence of S9 (Fig. 8a- e) was noticed. Significant differences ($p < 0.01$) were observed for TA97a, TA100 and TA104 (+S9 and -S9; Appendix 1f). For TA98, significant differences ($P < 0.001$) between treated and the DMSO control in an F-test, were only observed with S9 (Appendix 1f).

The extract was toxic in all three plates at the

Table 3f Mutagenicity of the methanol extract of *M. salicifolia* in the *Salmonella* tester strains.

Dose ug/plate	Revertants /plate (Mean ± S.D)[A]									
	TA97a		TA98		TA100		TA102		TA104[D]	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0[B]	238±2	169±34	28±3	35±3	91±23	221±15	319±35	320±14	396±14	395±7
62.5	312±12	372+4	32±4	263±40	120±36	426±7	327±30	319±3	405±74	442+13
125	360±60	379±26	27±5	308±8	129±7	443±40	322±24	319±4	433±73	459±20
250	319±20	427±30	28±2	308±10	102±2	466±40	327±30	329±28	726±103	840±20
500	247±49	415±5	27±8	303±55	130±14	463±8	316±14	319±3	630±70	831±29
1000	245±12	410±22	27±5	251±10	145±16	448±41	319±35	320±14	445±39	527±19
Positive control[C]	1536	1200	120	1084	220	690	307	324	-	-

A. Mean ± standard deviation oc counts from triplicates.
B. DMSO control value for the tester strain.
C. ICR - 191 served as positive control in the non-activated (-S9) test while 2 -AF served as positive control in the activated (+S9) test.
D. Positive controls not available for TA104.

Fig. 8

Mutagenic effect of the methanol extract of *M. salicifolia* in the *Salmonella* tester strains.
Each point represents the mean number of revertants in three plates after deduction of appropriate control values.
(f) -S9 and (g) +S9 show the summary effect of the extract in all tester strains.

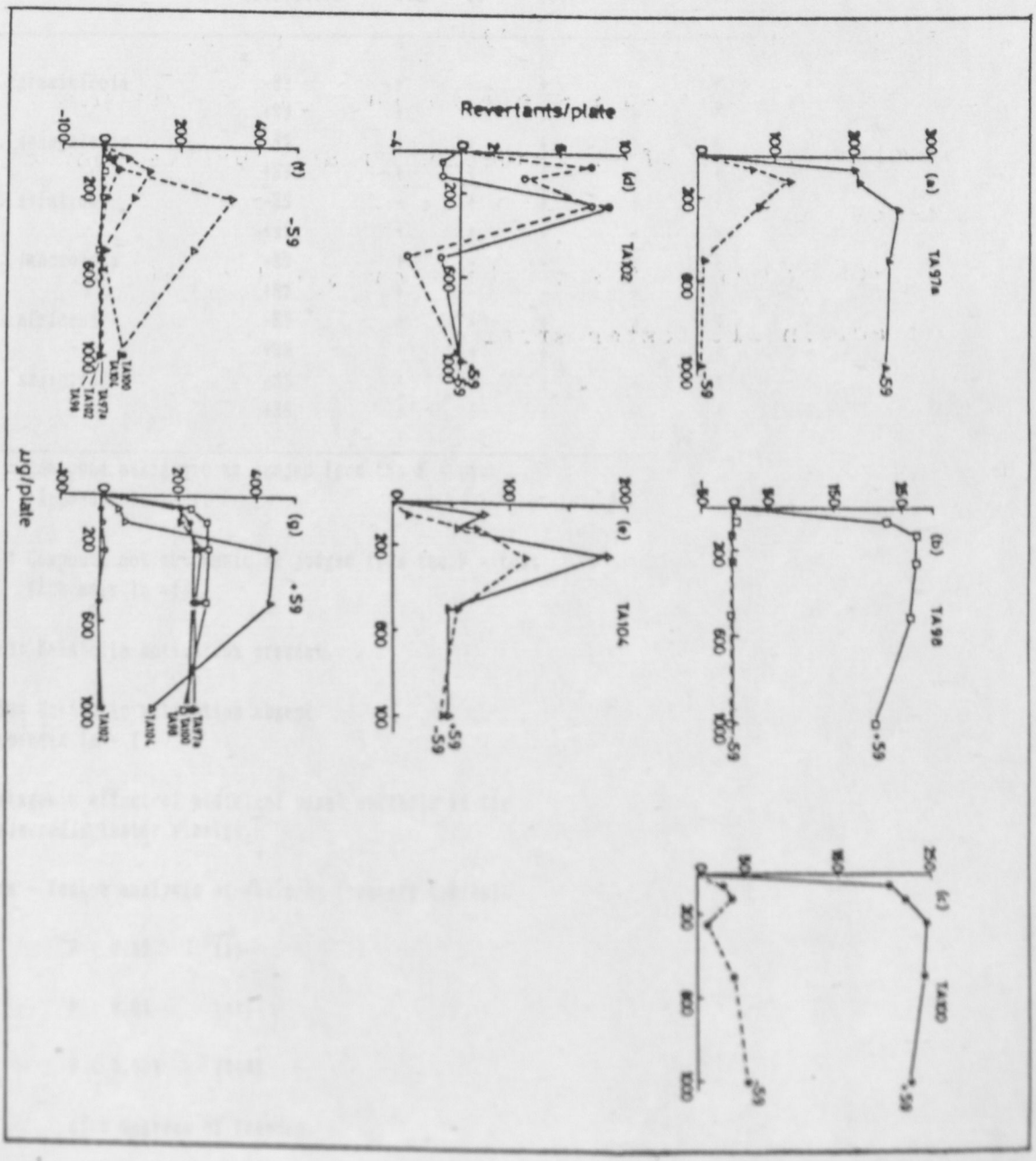

Revertants/plate
µg/plate
(a) TA97a
(b) TA98
(c) TA100
(d) TA102
(e) TA104
(f) -S9
(g) +S9

Fig. 8

Table 3g. Mutagenic effect of medicinal plant extracts in the *Salmonella* tester strains (overall summary of results).

Plant extract	Metabolic activation	Tester Strains 97a	98	100	102	104
A. graminicola	-S9	+	-	+	-	+
	+S9	+	-	+	+	+
A. senegalesis	-S9	+	+	+	+	+
	+S9	+	+	+	+	+
C. asiatica	-S9	-	+	+	+	+
	+s9	+	+	+	+	+
M. lanceolata	-S9	+	-	-	+	+
	+S9	+	-	-	+	+
M. africana	-S9	+	-	+	+	+
	+S9	+	+	+	-	+
M. salicifolia	-S9	+	-	+	-	+
	+S9	+	+	+	-	+

\+ = Compound mutagenic as judged from the F - test (Appendix 1a - f).

\- = Compound not mutagenic as judged from the F - test (Appendix 1a -f).

+S9= Metabolic activation present.

-S9= Metabolic activation absent.

Appendix 1a - f

Mutagenic effect of medicinal plant extracts in the *Salmonella* tester strains.

One - factor analysis of variance (summary tables).

P < 0.05 (*)

P < 0.01 (**)

P < 0.001 (***)

df = degrees of freedom.

last two doses of treatment in TA97a (+S9 and -S9). Small pin-point colonies were observed in these plates and occasional large colonies, which were regarded as revertants were also noticed. Toxicity was also observed in TA104 in the last two doses of treatment but here the colonies were bigger and the lawn was thinner as compared to the control.

The extract can be regarded to be most mutagenic in TA104 as compared to the other strains with and without S9 (Fig. 8f & g). It is only in TA104 was the mutagenic response significant in a t-test (P = 0.05; Appendix 2f).

Overall summary of mutagenic effects of medicinal plant extracts in the tester strains is presented in Table 3g.

3.2. Section 2 : *Vicia faba (V. faba)*

The plants evaluated for mutagenicity in the *Salmonella* test were tested for their ability to induce chromosomal damage in the root meristematic cells of *V. faba* using the method of Kihlman (1971). Details concerning treatment of the root meristems with the plant extract are described in Chapter 2. The results obtained are described below:

3.2.1. Abnormal metaphases.

These are described as those metaphases which looked condensed or sticky (Fig. 9a). Such metaphases were observed in roots treated with the methanol extract of *C. asiatica, M. salicifolia* and fresh sap of *A. graminicola* both in the roots fixed immediately after treatment and those allowed to recover. The extract of *A. senegalensis* produced abnormal metaphases only in roots fixed immediately after treatment.

C- metaphases (Fig. 9c) were recorded in roots treated with the methanol extract of *M. salicifolia*. No abnormal metaphases were observed in roots treated with the methanol extract of *M. lanceolata*. Statistical analysis of the data (Appendix 4a) revealed that there was enhancement in the production of abnormal metaphases in treated roots over the control in a dose related fashion. Thus in all cases there was a 95% real correlation [$P < 0.05$; Fig. 12(i)] between treatment and production of abnormal metaphases in the roots fixed immediately after treatment. Significant results, $P < 0.05$ in roots allowed to recover was observed in *C. asiatica and M. africana* (Fig. 12ii).

Although abnormal metaphases were induced in roots treated with fresh sap of *A. graminicola* and recovered for 20 hours, their production was not signi-significant in the F-test [$P > 0.05$; Fig 12 (iii)].

Fig. 9

Photomicrographs showing abnormal metaphases after treatment of *V. faba* with some of the plant extracts

(a) A sticky metaphase observed in *V. faba* root meristem cells treated for 2 hours with 5 mg/ ml od the methanol extract of *M. africana* (X1200).
(b) A normal metaphase observed in *V. faba* root meristem cells from the control. (X1200).
(c) A C-metaphase observed in *V. faba* root meristem cells treated for 2 hours with 10 mg/ml of the methanol extract of *M. salicifolia* (X1200).
(d) A C-metaphase observed in *V. faba* root meristem cells treated for 2 hours with 5 mg/ml of the methanol extract of *M. salicifolia* (X1200)
(e) A normal metaphase observed in *V. faba* root meristem cells from the control (X1200).

⟶ indicate gaps.

Fig. 9

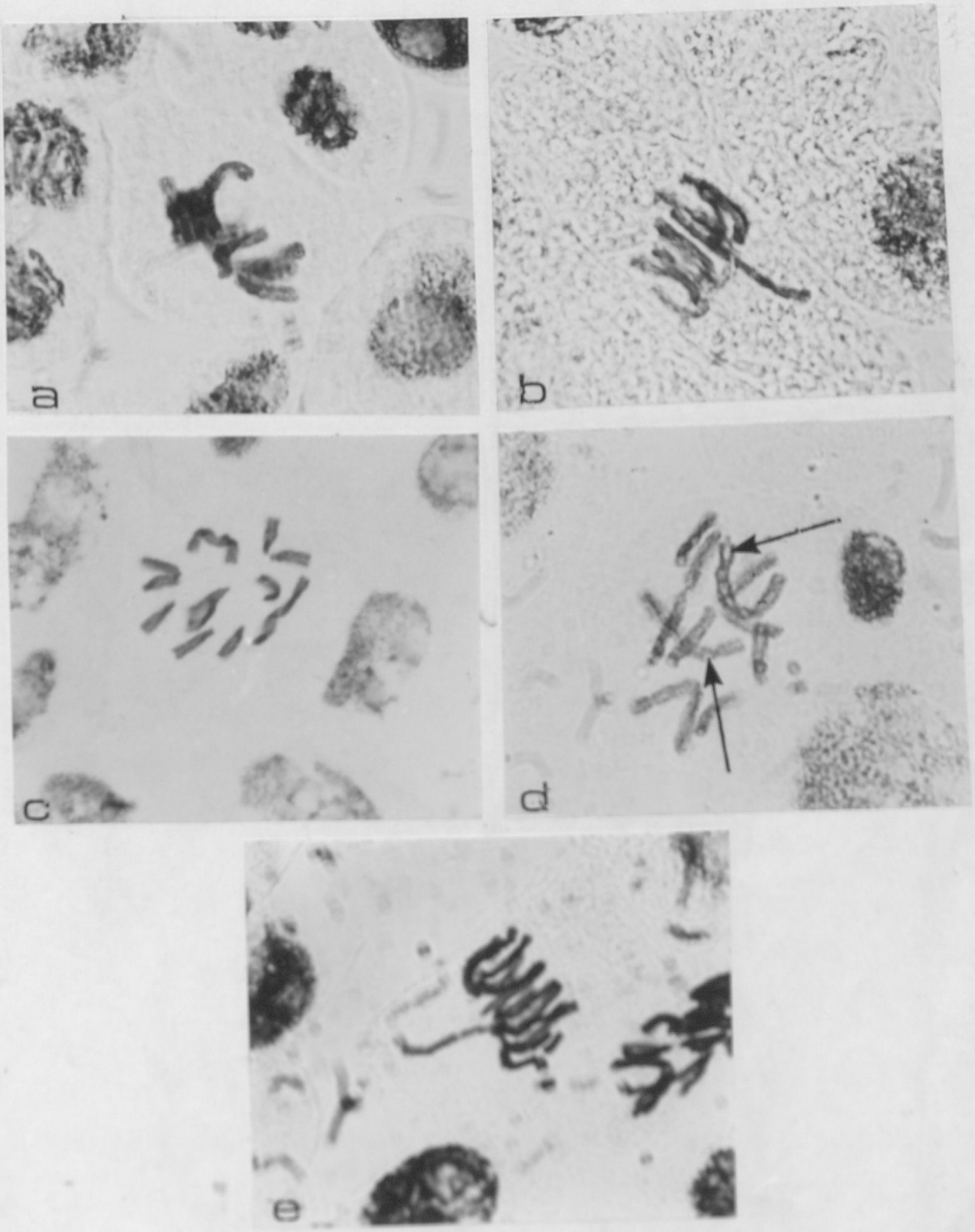


Fig. 10

A photomicrograph showing disrupted spindle apparatus at anaphase

(a) An anaphase with disrupted spindle apparatus observed in *V. faba* root meristem cells treated for 2 hours with 10 mg/ml of the methanol extract of *M. salicifolia* (X1200).

(b) A normal anaphase observed in *V. faba* root meristem cells from the control (X1200)

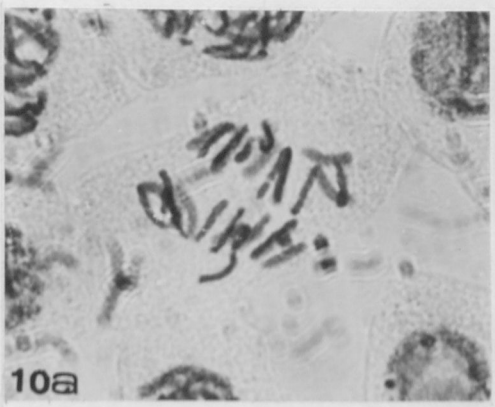
Fig. 11

A photomicrograph showing prophase bridges

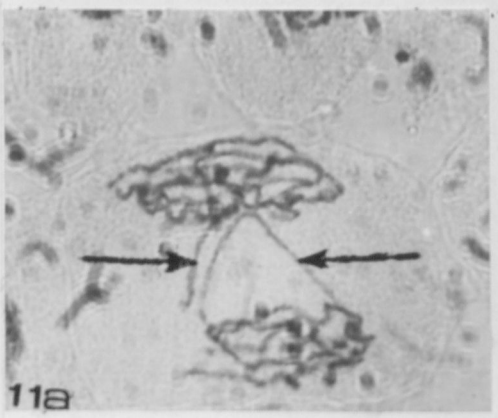
(a) Prophase bridges observed in *V. faba* root meristem cells treated for 2 hours with 10 mg/ml of the methanol extract of *M. salicifolia* (X1200).

(b) A normal prophase observed in *V. faba* root meristem cells from the control (X1200).

⟶ indicates bridge .


10a


10b


11a

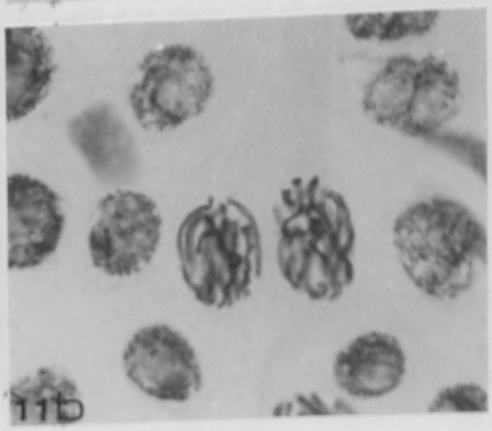

11b

Fig. 12(i)

Relationship between production of abnormal metaphases in *V. faba* root meristem cells and treatment for 2 hours with various plant extracts

A. (—·—) *A. senegalensis*, y = 4.18 + 1.66x, P < 0.05, r = 0.9491.

B. (—), *C. asiatica*, y = 5.25 + 1.16x, P < 0.05, r = 0.08857.

C. (- - -) *M. africana*, y = 0.75 + 2.046x, P < 0.05 r = 0.9419

D. (— ·) *M. salicifolia* y = -0.725 +4.14x, P < 0.05, r = 0.9525.

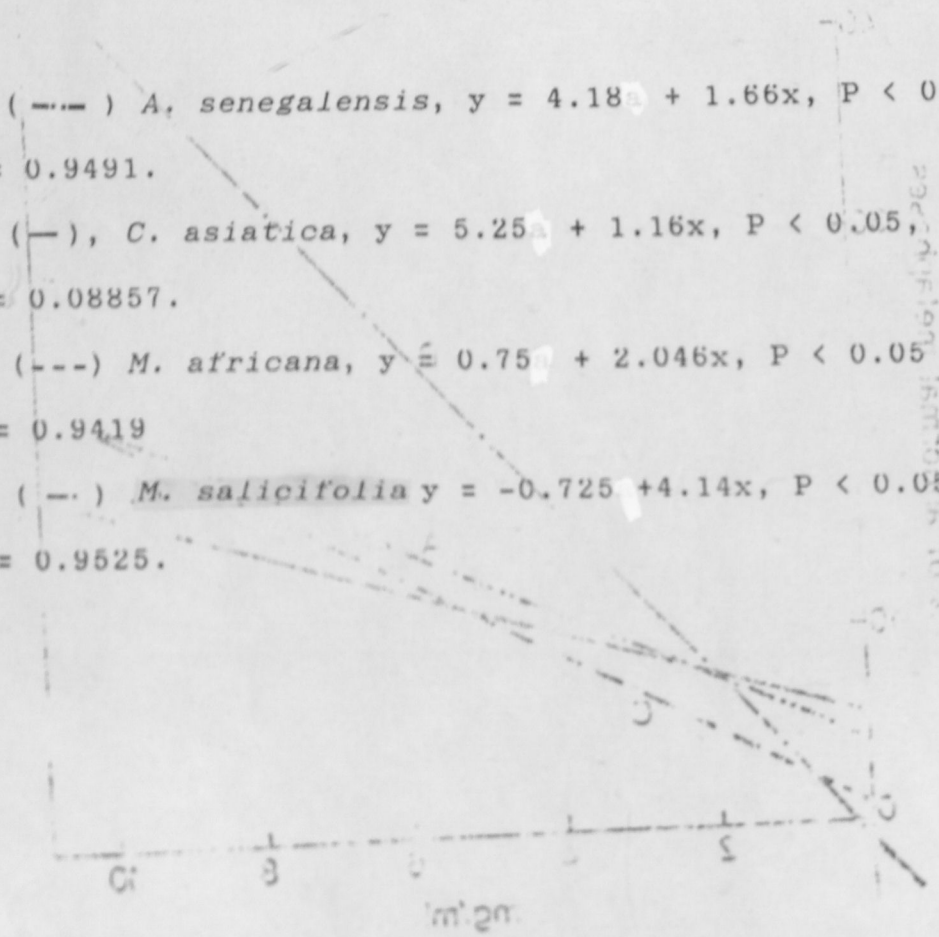


Fig. 12(i)

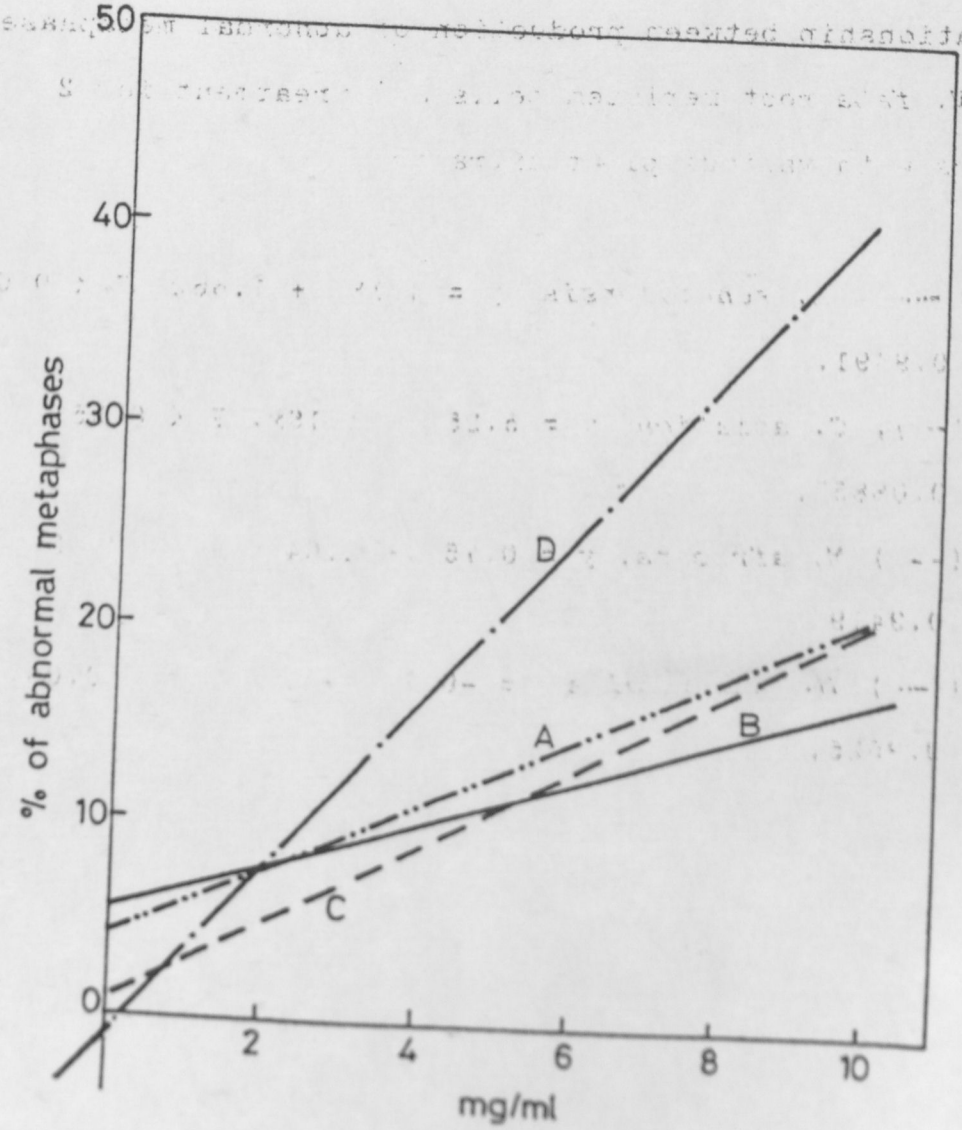


Fig. 12(ii)

Relationship between production of abnormal metaphases in *V. faba* root meristem cells and treatment with the various plant extracts for 2 hours and allowed to recover for 20 hours.

B. (—) *C. asiatica*, y = -1.38 + 1.22x, P < 0.05, r = 0.9513.

C. (---) *M. africana*, y = 0.77 + 0.7x, P > 0.05, r = 0.8622.

D. (—·) *M. salisfolia*, y = 5.53 + 3.86x, P < 0.05, r = 0.8937.

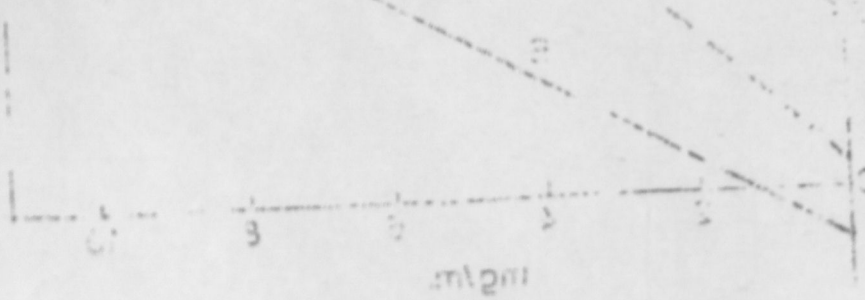


Fig. 12(ii)

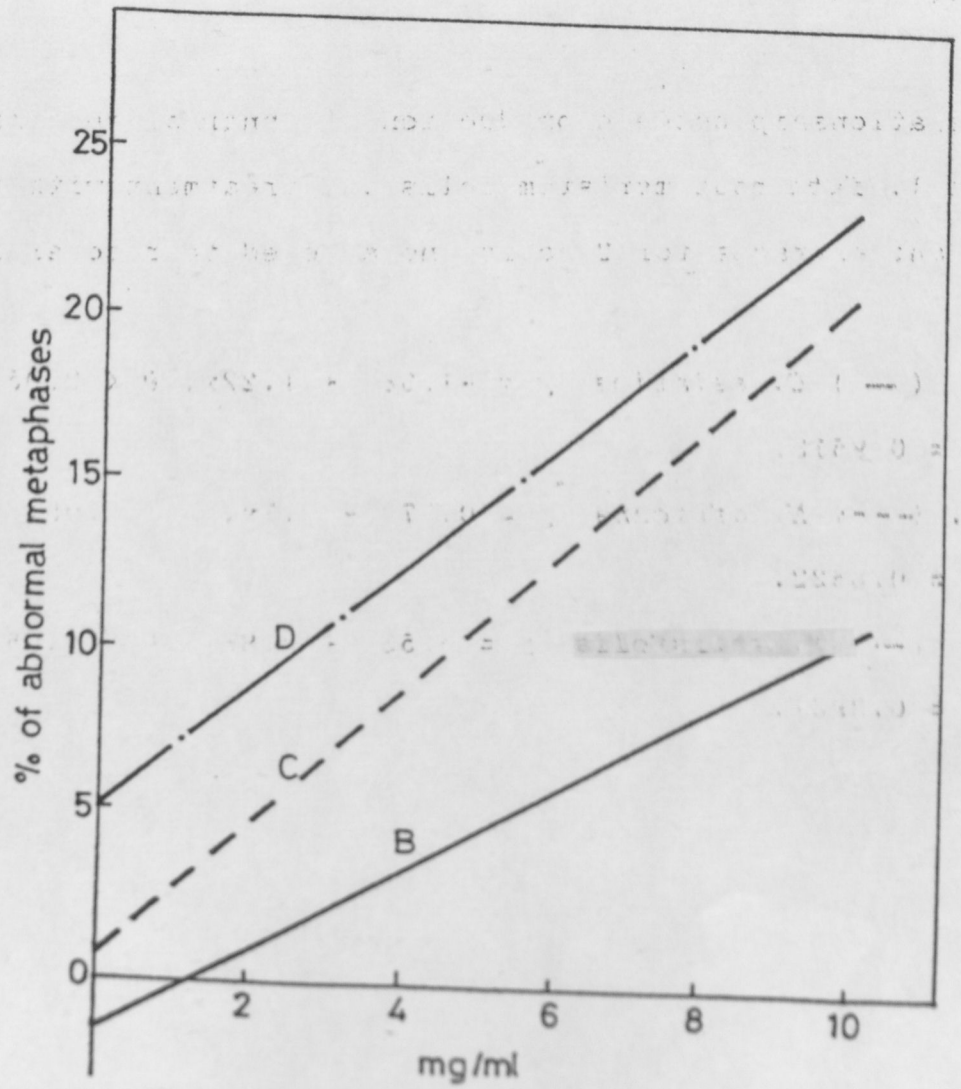


Fig. 12(iii)

Relationship between production of abnormal metaphases and treatment of *V. faba* root meristem cells with the fresh sap of *A. graminicola*.

A. (▲) roots fixed immediately after treatment,

$y = 2.975 + 1.02x$, $P < 0.05$, $r = 0.9324$.

B. (△) roots allowed to recover for 20 hours,

$y = 5.15 + 2.52x$, $P > 0.05$, $r = 0.8125$.

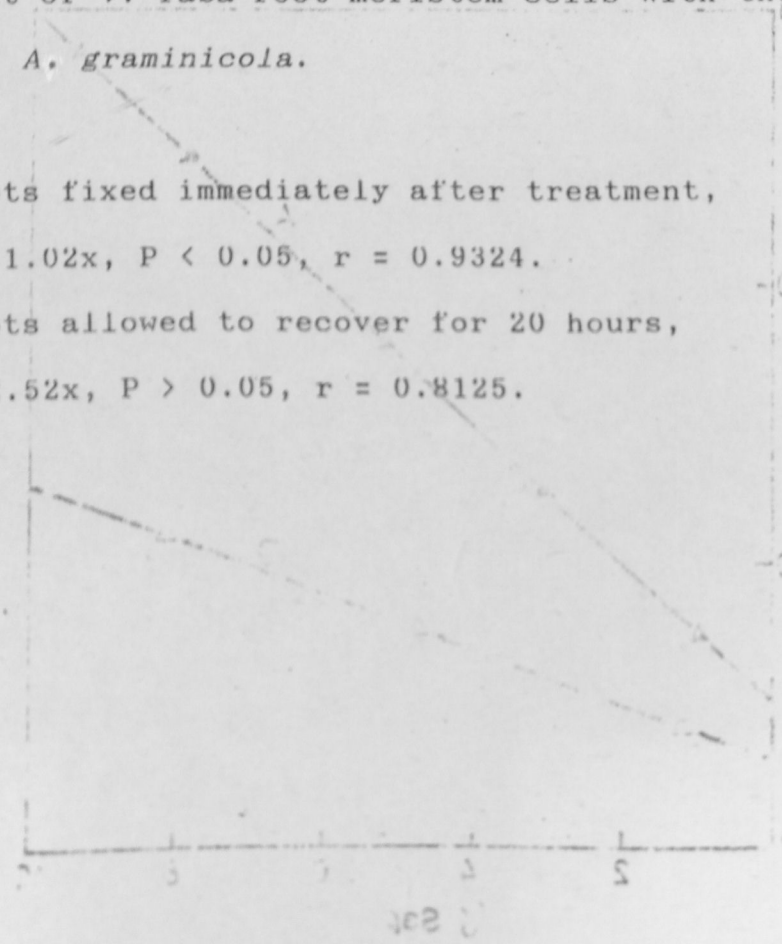
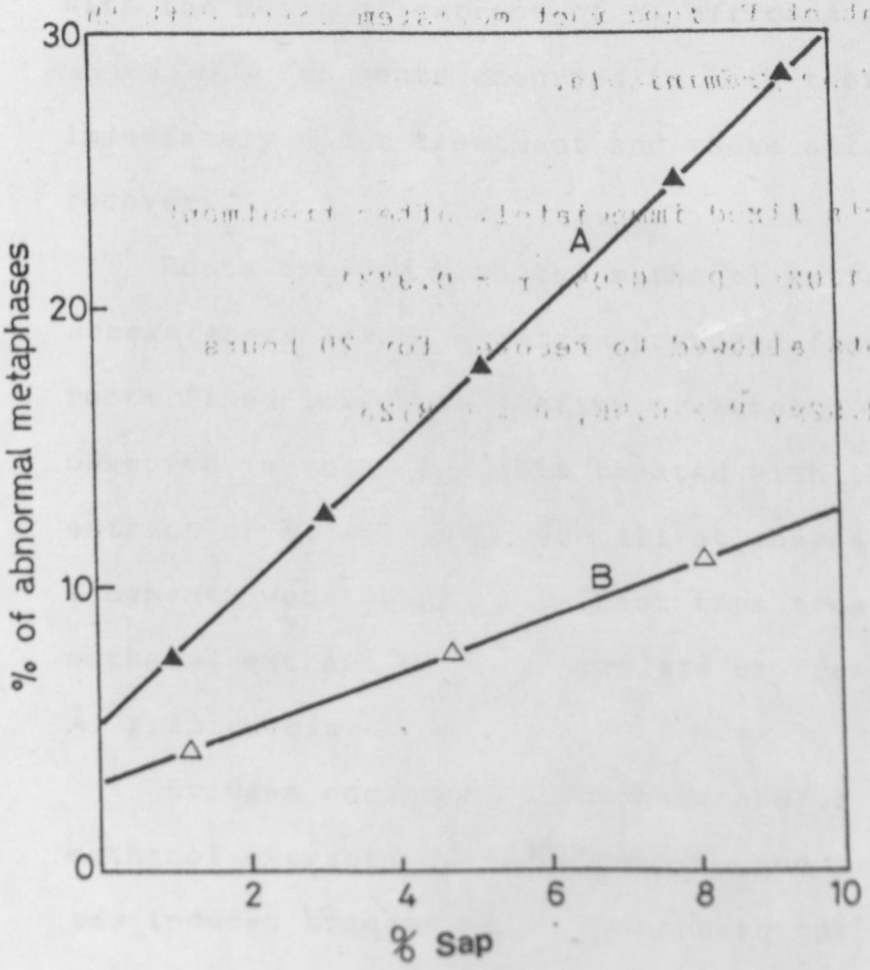


Fig. 12 (iii)



3.2.2. Chromosomal aberrations

Chromosomal aberrations were observed as bridges and fragments (Fig. 13 & 14). Fragments were recorded in metaphase and anaphase chromosomes. In roots treated with the methanol extract of *M. africana* and *M. salicifolia* fragments occurred in both roots fixed immediately after treatment and those allowed to recover.

Roots treated with the methanol extract of *A. senegalensis* and *C. asiatica* produced fragments only in roots fixed immediately after treatment. The fragments observed in root tip cells treated with the methanol extract of *M. africana* were all at anaphase. No fragments were observed in root tips treated with the methanol extract of *M. lanceolata* or fresh sap of *A. graminicola*.

Bridges occurred at anaphase and/or telophase. The methanol extracts of *M. salicifolia* and *A. senegalensis* induced bridges only in anaphase chromosomes while the rest of the plant extracts induced bridges both in telophase and anaphase. Occasionally more than one bridge occurred in anaphase (Fig. 13b).

The methanol extracts of *M. africana* and *M. salicifolia* induced chromosomal aberrations in *V. faba* root tips, hence, a strong correlation ($r = 0.9776$ for *M. africana* and 0.9600 for *M. salicifolia*) in the roots fixed

Fig. 13

Photomicrographs showing chromosomal bridges after treatment of *V. faba* with some of the plant extracts

(a) An anaphase bridge observed in *V. faba* root meristem cells after treatment for 2 hours with 2.5 mg/ml of the methanol extract of *A. senegalensis* (X1200)

(b) An anaphase showing 2 bridges after treatment of *V. faba* root meristem cells with the methanol extract of *M. salicifolia* (X1200).

(c) A normal anaphase observed in *V. faba* root meristem cells from the control (X1200).

(d) A bridge observed in telophase of *V. faba* root meristem cells after treatment for 2 hours with 10% sap of *A. graminicola* (X1200).

(e) A normal telophase observed in *V. faba* root meristem cells from the control (X1200).

→ indicates bridge.

Fig.13

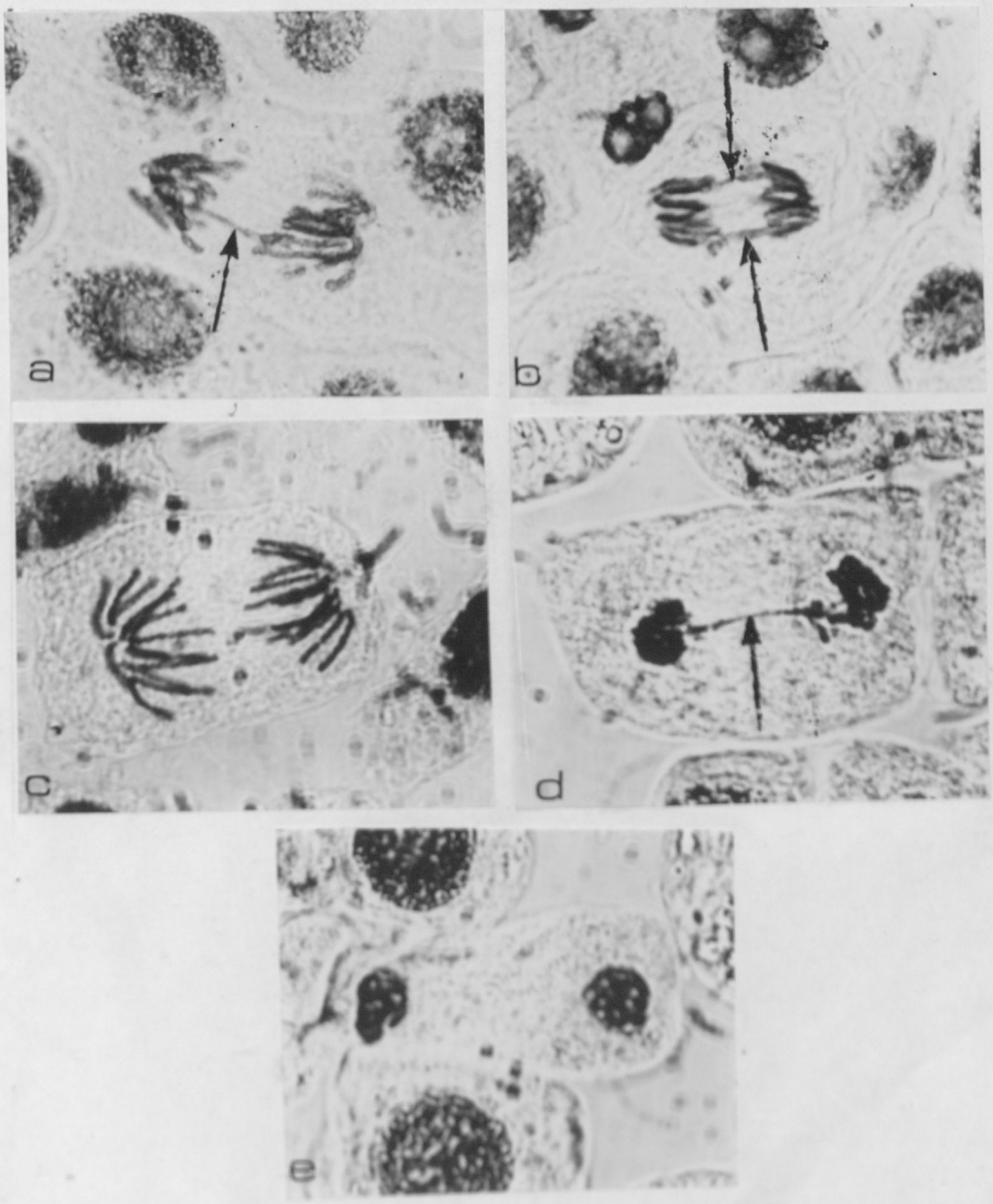
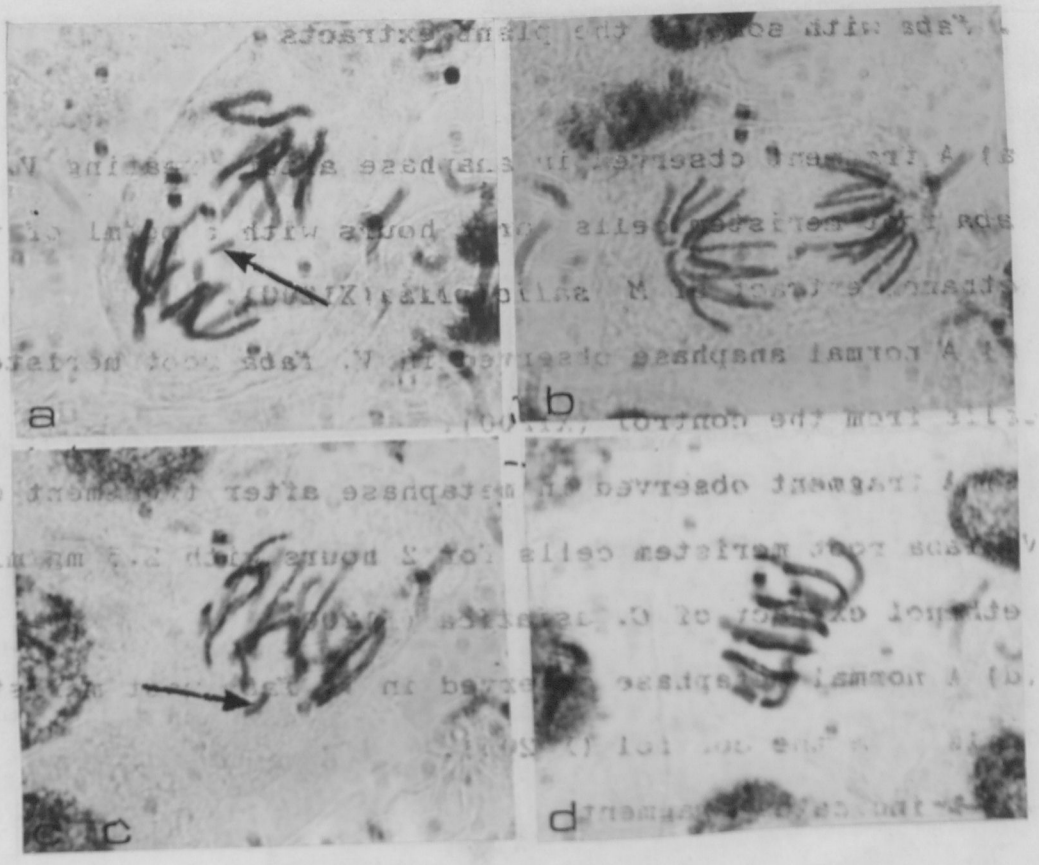


Fig . 14

Photomicrographs showing fragments after treatment of *V. faba* with some of the plant extracts

(a) A fragment observed in anaphase after treating *V. faba* root meristem cells for 2 hours with 5 mg/ml of the methanol extract of *M. salicifolia*(X1200).
(b) A normal anaphase observed in *V. faba* root meristem cells from the control (X1200).
(c) A fragment observed in metaphase after treatment of V. faba root meristem cells for 2 hours with 2.5 mg/ml methanol extract of *C. asiatica* (X1200).
(d) A normal metaphase observed in *V. faba* root meristem cells from the control (X1200).
⟶ indicates fragment

Fig.14



immediately after treatment, and a correlation of 0.9347 for *M. africana* and 0.9200 for *M. salicifolia* in the roots allowed to recover. This enhancement is also reflected in the regression anova [Fig. 15(i) Appendix 3] where the regression coefficient b was found to be significant ($P < 0.05$).

The methanol extract of *C. asiatica* induced chromosomal aberrations in the roots fixed immediately after treatment and those allowed to recover but no significant relationship was observed between production of aberrations and treatment [$P > 0.05$; Fig. 15(i) & (ii)]. The methanol extract of *A. senegalensis* significantly induced chromosomal aberrations only in roots allowed to recover but not in those fixed immediately after treatment [Fig. 15(i) & (ii)].

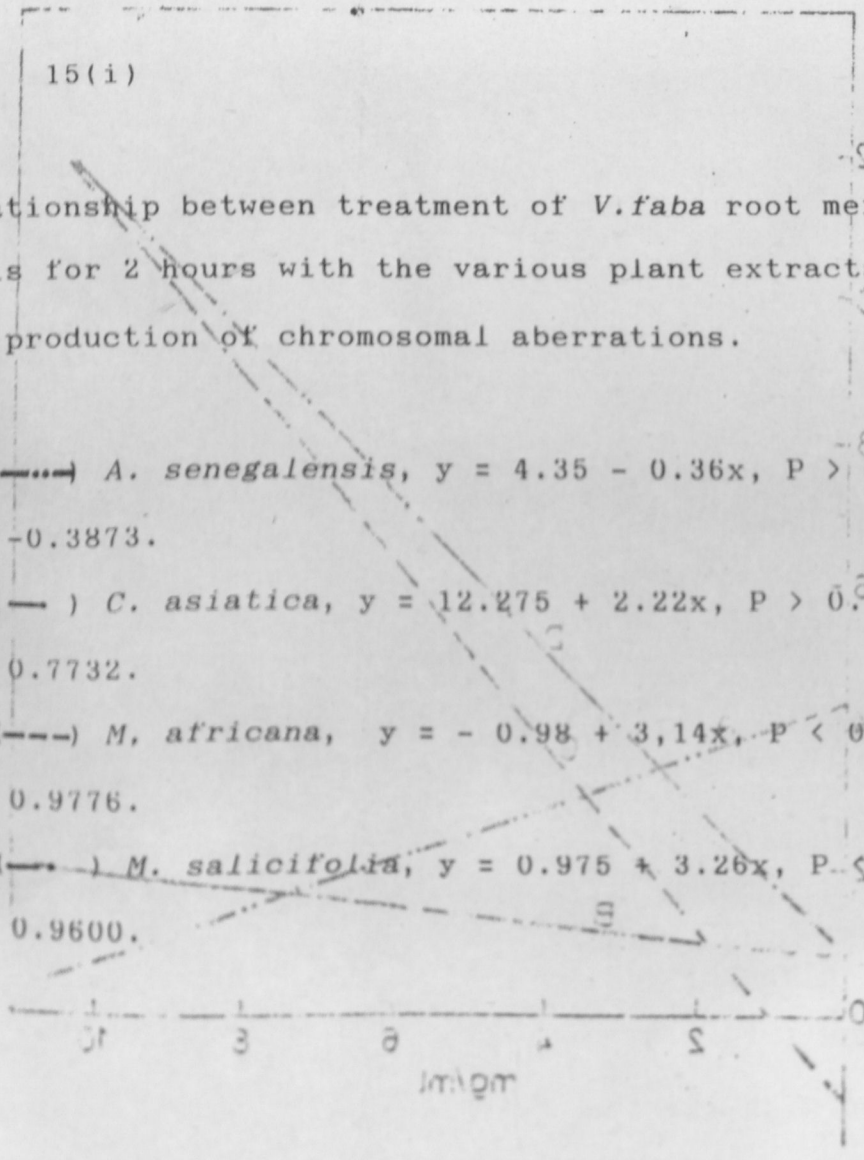
3.2.3. Micronuclei (MCN)

MCN (Fig. 16) were only observed in those roots treated with the fresh sap of *A. graminicola* and allowed to recover. There was a strong correlation ($r = 0.9625$) between number of cells with micronuclei and the dose of treatment (Fig. 17).

3.2.4. Enlarged nucleoli

Enlarged nucleoli were a common feature in roots treated with the methanol extract of *M. lanceolata* and

Fig. 15(i)



Relationship between treatment of *V.faba* root meristem cells for 2 hours with the various plant extracts and the production of chromosomal aberrations.

A. (—·—·—) *A. senegalensis*, y = 4.35 - 0.36x, P > 0,05, r = -0.3873.

B. (——) *C. asiatica*, y = 12.275 + 2.22x, P > 0.05, r = 0.7732.

C. (— — —) *M. africana*, y = - 0.98 + 3,14x, P < 0.05, r = 0.9776.

D. (— · —) *M. salicifolia*, y = 0.975 + 3.26x, P < 0.05, r = 0.9600.

Fig. 15(i)

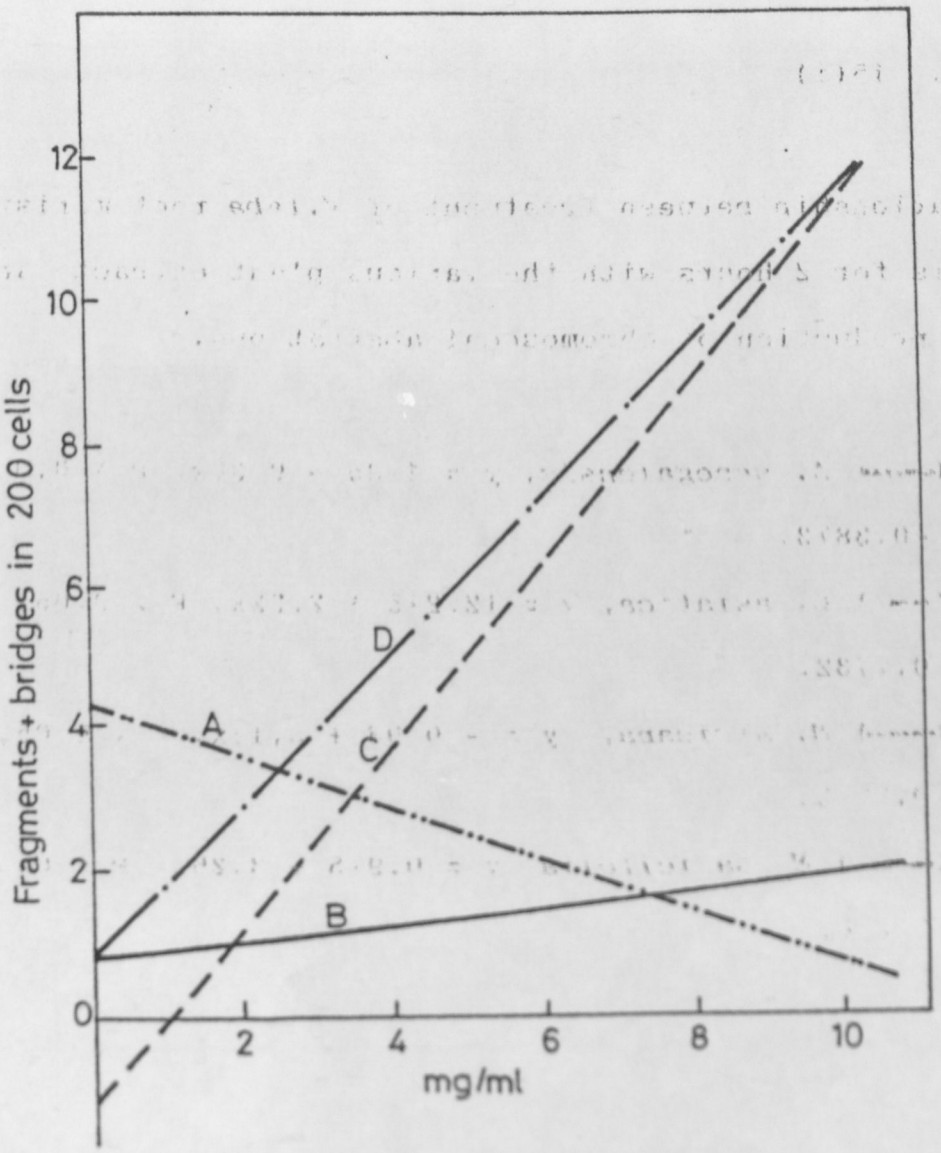


Fig. 15(ii)

Relationship between treatment of *V. faba* root meristem cells for 2 hours with the various plant extracts and production of chromosomal aberrations in roots allowed to recover for 20 hours.

A. (—·—) *A. senegalensis*, y = 3.05 + 2.76x, P < 0.05, r = 0.9670.

B. (——) *C. asiatica*, y = 0.75 + 0.12x, P > 0.05, r = 0.5669.

C. (— — —) *M. africana*, y = -0.14 + 1.28x, P < 0.05 r = 0.9347.

D. (——·) *M. salicifolia*, y = 0.83 +1.06x, P < 0.05, r = 0.9209.

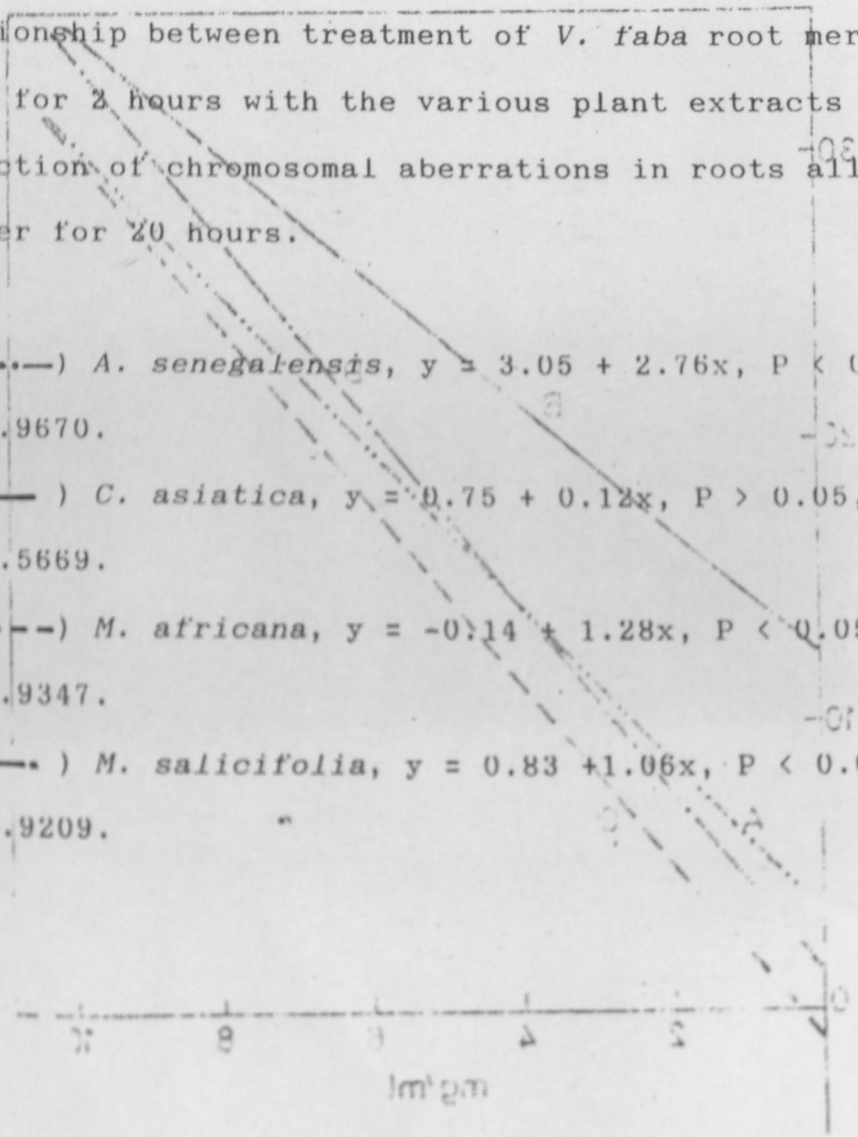


Fig. 15(ii)

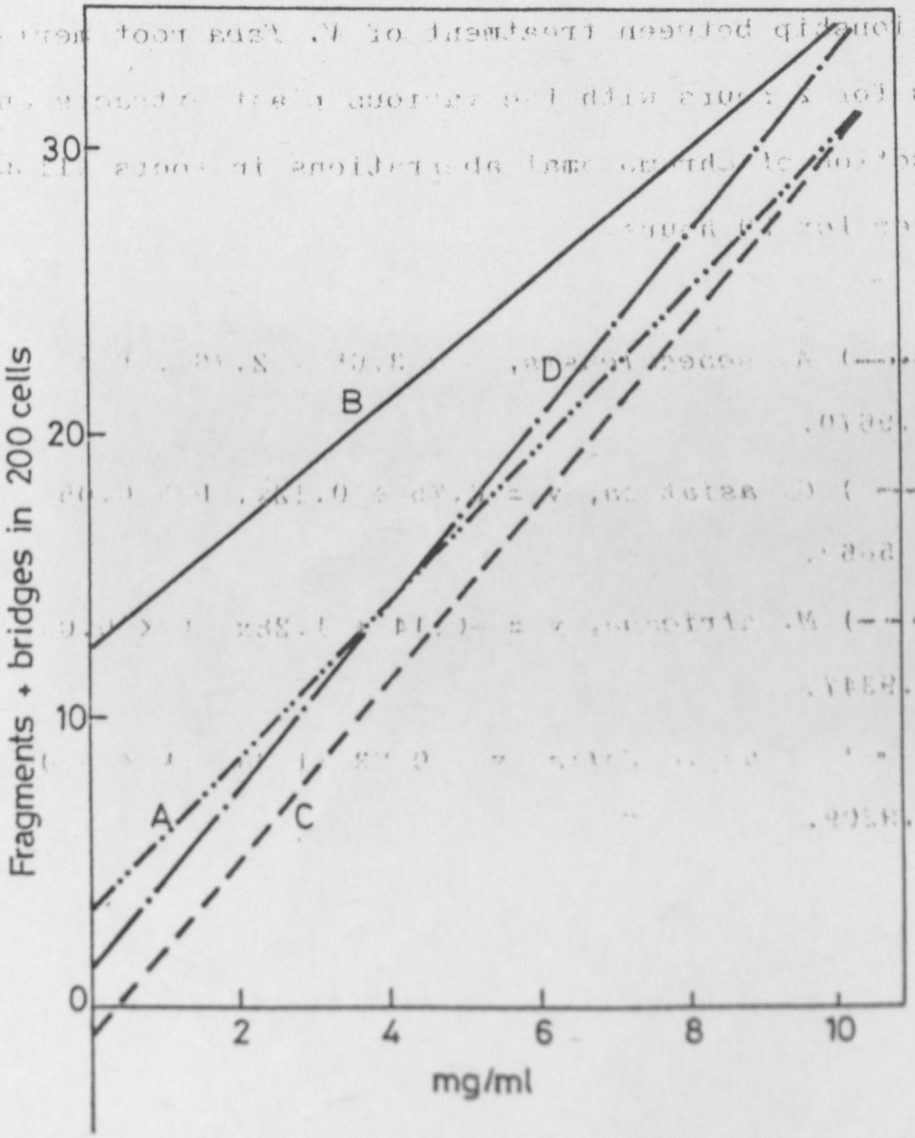


Fig. 16

Photomicrographs showing micronuclei (MCN) after treatment of *V. faba* with the sap of *A. graminicola*

(a) 2 MCN attached on an interphase nucleus of *V. faba* root meristem cells treated with 5% sap of *A. graminicola* and recovered for 20 hours (X1200).

(b) An (MCN) observed at interphase in *V. faba* root meristem cells treated for 2 hours with 10% sap of *A. graminicola* and allowed to recover for 20 hours (X1200).

→ indicates the MCN.

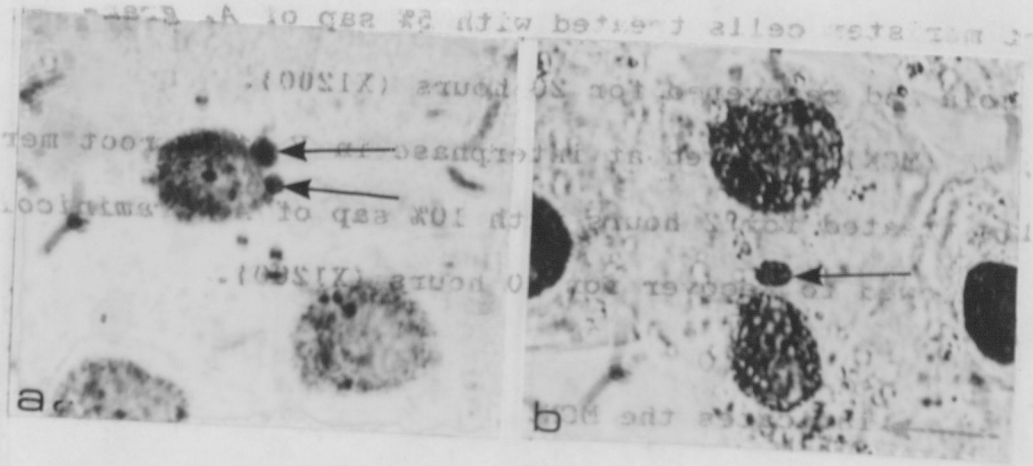


Fig. 16

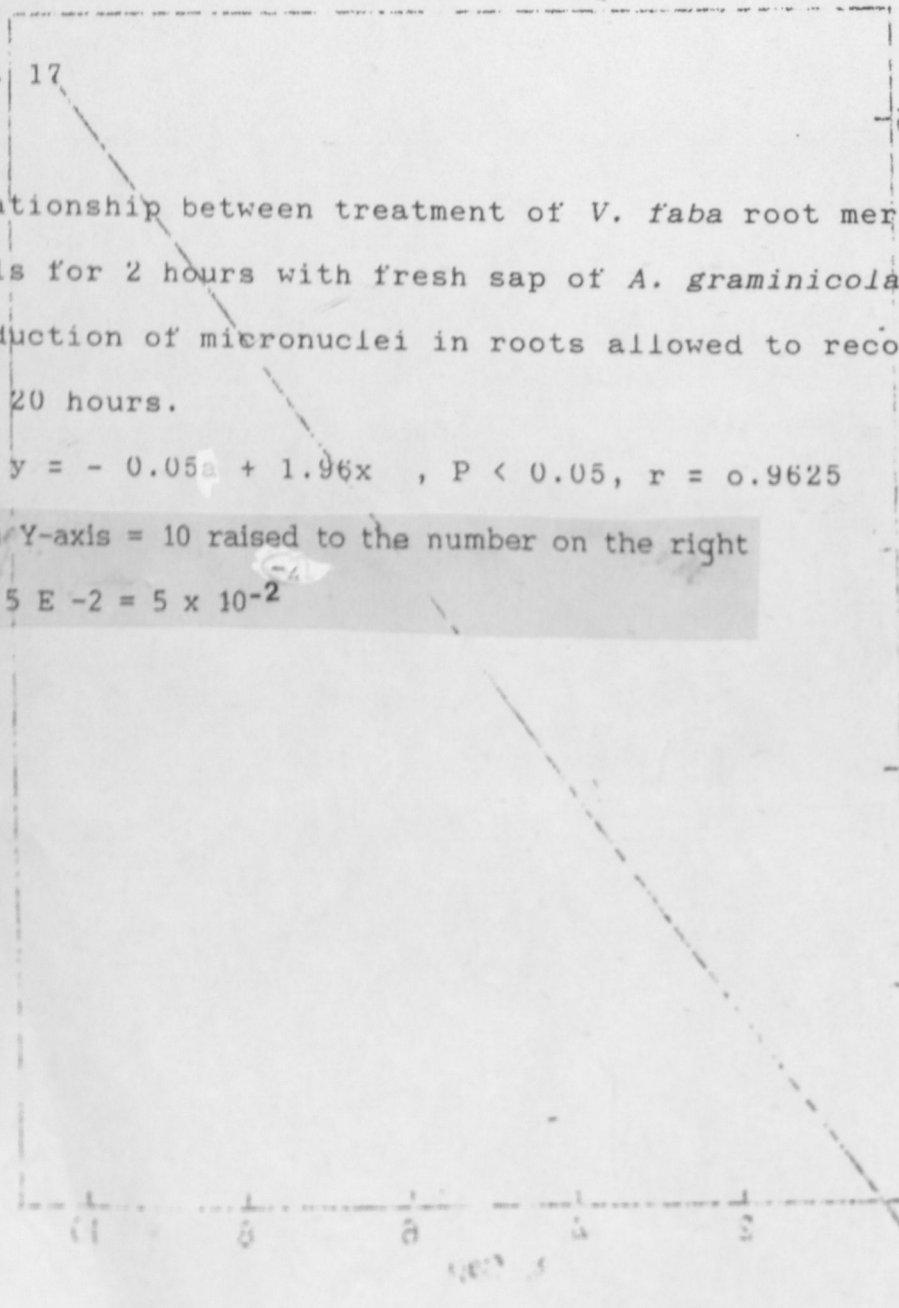


Fig. 17

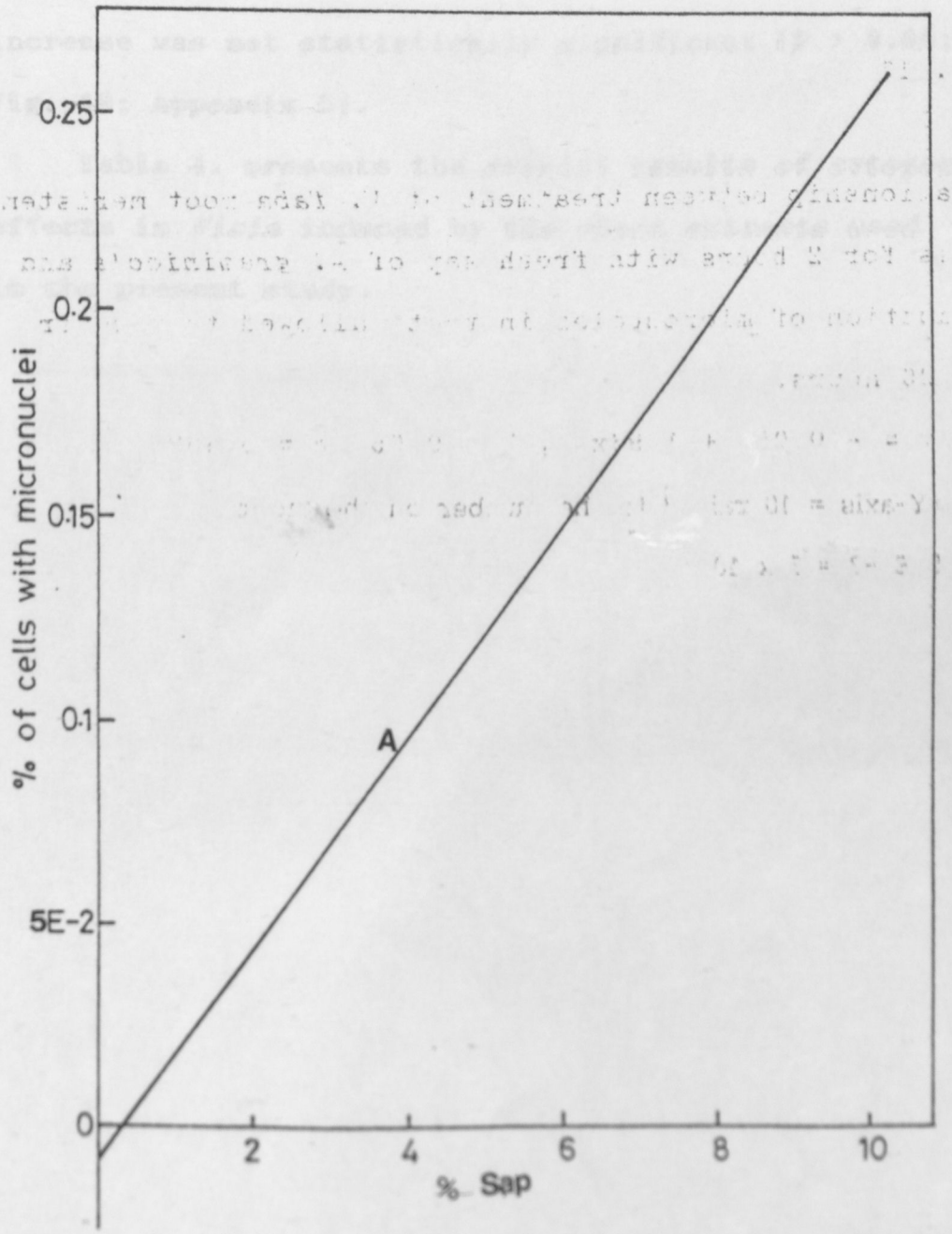
Relationship between treatment of *V. faba* root meristem cells for 2 hours with fresh sap of *A. graminicola* and the production of micronuclei in roots allowed to recover for 20 hours.

A. $y = -0.05a + 1.96x$, $P < 0.05$, $r = 0.9625$

E on Y-axis = 10 raised to the number on the right

e.g. 5 E -2 = 5×10^{-2}

Fig. 17



recovered for 20 hours (Fig 18a). There was an apparent increase in the volume of the nucleoli, however, this increase was not statistically significant [$P > 0.05$; Fig. 19; Appendix 5].

Table 4. presents the overall results of cytogenetic effects in *Vicia* induced by the plant extracts used in the present study.

recovered for 20 hours (Fig 18a). There was an apparent increase in the volume of the nucleoli, however, this increase was not statistically significant [$P > 0.05$; Fig. 19; Appendix 5].

Table 4. presents the overall results of cytogenetic effects in *Vicia* induced by the plant extracts used in the present study.

Fig. 18

A Photomicrograph showing enlarged nucleoli after treatment of *V. faba* with the methanol extract of *M. lanceolata*

(a) Interphase cells showing enlarged nucleoli in roots treated for 2 hours with 5 mg/ml of the methanol extract of *M. lanceolata* and allowed to recover for 20 hours (X1200).

(b) A normal interphase nucleus from the control (X1200).

⟶ indicate nucleoli.

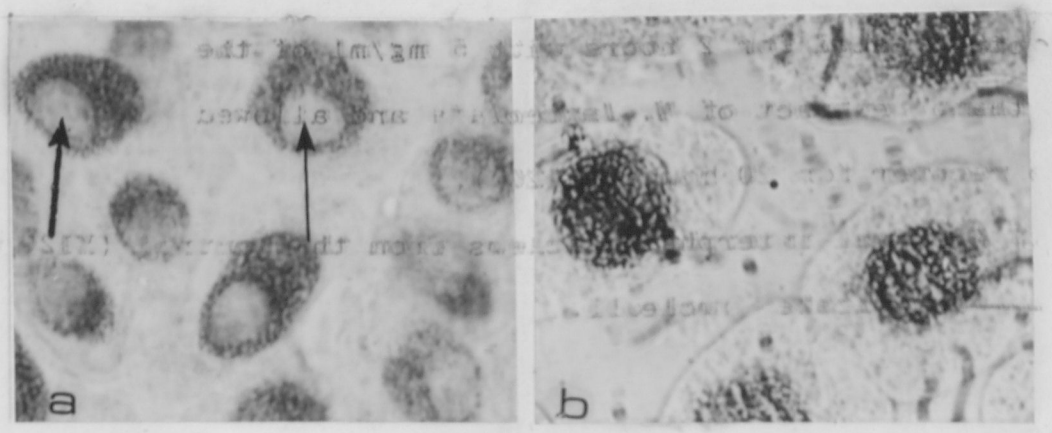
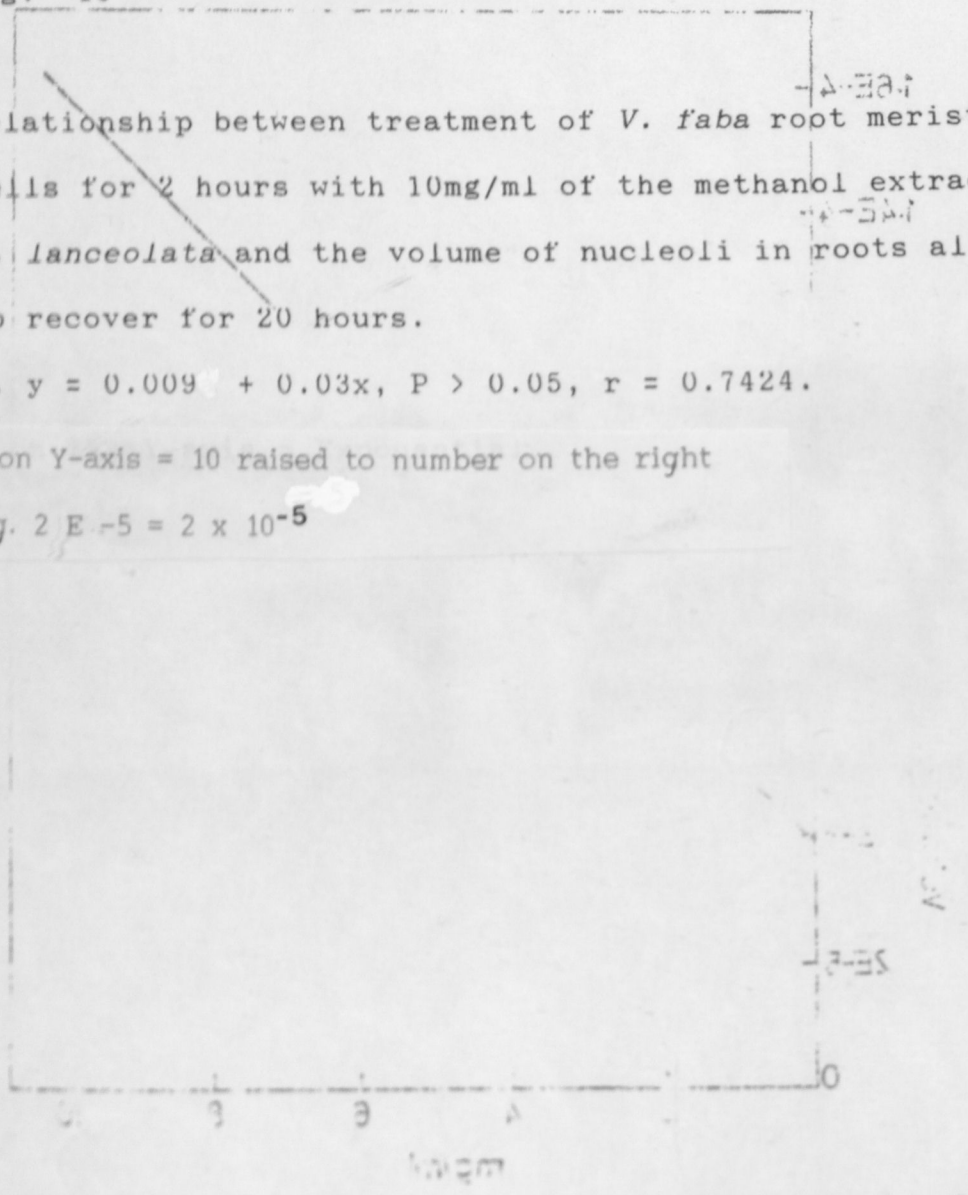


Fig. 18

Fig. 19



Relationship between treatment of *V. faba* root meristem cells for 2 hours with 10mg/ml of the methanol extract of *M. lanceolata* and the volume of nucleoli in roots allowed to recover for 20 hours.

A. $y = 0.009 + 0.03x$, $P > 0.05$, $r = 0.7424$.

E on Y-axis = 10 raised to number on the right

e.g. 2 E -5 = 2×10^{-5}

Fig. 19

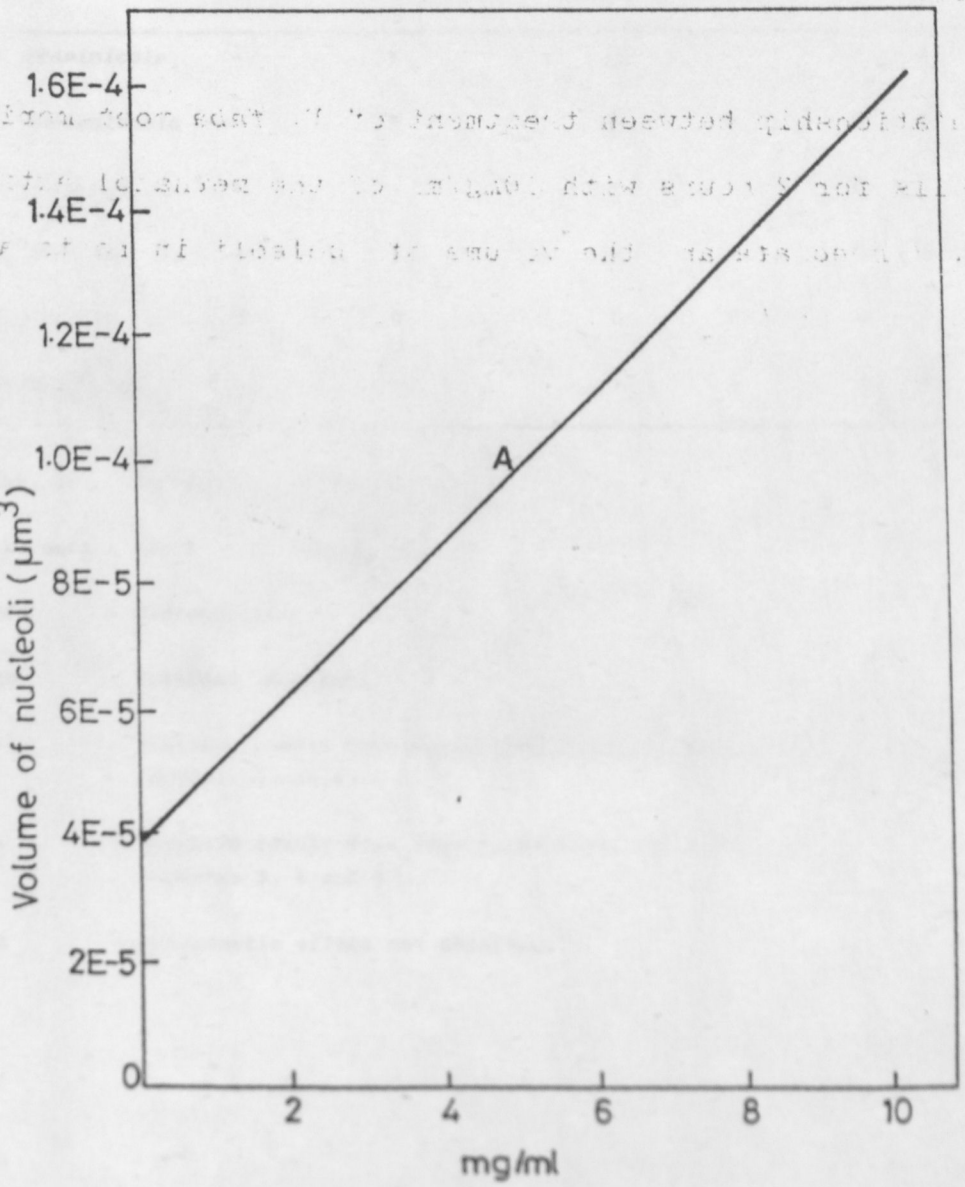

1·6E-4
1·4E-4
1·2E-4
1·0E-4
8E-5
6E-5
4E-5
2E-5
0
Volume of nucleoli (μm³)
A
2
4
6
8
10
mg/ml

Table 4. *Vicia* cytogenetic test results.

Plant extract	Non Recovered		Recovered			
	Chr. ab	Ab. Meta.	Chr. ab.	Ab. meta.	MCN	EN.
A. graminicola	-	+	0	-	+	0
A. senegalensis	-	+	+	0	0	0
C. asiatica	-	+	-	+	0	0
M. africana	+	+	+	-	0	0
M. lanceolata	0	0	0	0	0	-
M. salicifolia	+	+	+	+	0	0

Chr. ab. = Chromosomal aberrations.

Ab. meta = Abnormal metaphases.

MCN = Micronuclei.

EN = Enlarged nucleoli.

\+ = Positive result from regression anova (P 0.05, Appendix 3 and 4).

\- = Negative result from regression anova (P 0.05, Appendix 3, 4 and 5).

0 = Cytogenetic effect not observed.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

4.1. Section 1 - *Salmonella*

In Table 3g, it can clearly be seen that crude extracts of medicinal plants used in this study exhibited mutagenic activity in the *Salmonella* test. A similar finding was made by Morimoto *et al.*, (1982) for water and methanol extracts obtained from crude drugs commonly used in Japan. Although the mutagenic compounds in these plants is not known at this stage, it has been reported elsewhere that components isolated from plants such as flavonoids (Brown, 1980), xanthone derivatives (Morimoto *et al.*, 1983; Kanamori *et al.*, 1984), anthraquinone derivatives (Brown *et al.*, 1979; Tikkanen *et al.*, 1983) and several pyrrolizidine alkaloids (Wehner *et al.*, 1979; Yamanaka *et al.*, 1979) exhibit mutagenic activity in the *Salmonella* test.

Mutagenic activity produced by the plant extracts was more with S9 than without it (Fig. 3-8). These results are supported by the findings of Mizuta and Kanamori (1985) who showed that mutagenic activity of dictamine and Y- fagarine from *Dictamni radicis cortex* (used as ingredient of Chinese medicines) increased with S9. An exception to this observation was made for the methanol

extract of *A. graminicola* in TA97a and TA100 (Fig. 3a) A similar observation was made for the methanol extract of *A. senegalensis* in TA100 (Fig 4c). In these observations there is a possibility that the S9 deactivated the mutagenic compounds into nonmutagenic ones. Such deactivation of mutagenic compounds in crude coffee has also been observed in *Salmonella* TA100 by Aeschbacher *et al.*, (1980).

The tester strain TA104 best detected mutagenicity in most of the plant extracts (Fig. 3-8) and this finding agrees with that of Marnett *et al.*, (1985) that TA104 is more sensitive than other strains for the identification of naturally occuring carbonyl compounds such as aldehydes and ketones as mutagens. These compounds may be occuring in the plant extracts used in this study.

The sensitivity of TA104 may be due to the presence of a nonsense mutation "AT" at the site of reversion on the hisG428 mutation along the histidine biosynthetic pathway. This mutation also occurs in TA102 (Levin *et al.*, 1982b) and these two strains are suitable for the detection of lesions induced by free radical generating agents. Therefore, greater effectiveness of TA104 over TA102 may be due to the presence of *uvrB* mutation in TA104 and its absence in TA102 (Table 2).

In general, the Ames minimal battery (of the strains TA97a, TA98, TA100 and TA102) and TA104 have proved an indispensable tool in the detection of medicinal plant

extracts as a potential source of mutagens. If TA98 and TA100 (Table 3g.) had been used alone to detect potential mutagens in the extract of *M. lanceolata*, one would have arrived at the conclusion that it is not mutagenic hence giving false negative results.

It might be argued that plant extracts containing histidine might give false positive results. However, in this study, mutagenicity of some of the plant extracts was negative in some of the tester strains Table (3g) and it would be expected that if histidine influenced positive results, this would have been expected for all strains. One exception is the methanol extracts of *A. sengal-ensis* which showed positive mutagenic response in all strains (Table 3g). But even for this extract, histidine could not have influenced these results since the highest number of revertants recorded was for TA104 (+S9; Fig. 4g) per 1000ug per plate and this number is too low compared to 18,250 reverants induced by methylglyoxal in TA104 (-S9; Marnett *et al.* 1985). The numbers that would have been expected if histidine influenced these results would even be higher. Approximately 1-2 x 10^8 cells are plated during mutagenicity assay and if histidine is available, each cell is expected to form a colony and no lawn would have been observed since all of them would have had a chance to grow to many tiny colonies.

One limitation in the detection of mutagens with some

of the plant extracts is the reduction of the number of colonies at higher doses. This could in part be attributed to toxic effects as seen with the methanol extract of *M. africana* in TA102 and TA104. The extract of *M. salicifolia* in TA97a and TA104 was also toxic Toxic effects were indicated by partial clearing of the background lawn in the affected plates as compared to the controls. In other cases, reduction in the number of revertants at higher doses could possibly be attributed to such unexplained factors like induction of DNA repair enzymes, solubility, saturation effects (Myers *et al.*, 1981), and changing the length of time the chemical was active in the petri dish (Margolin *et al.*, 1981).

4.2. Section 2 - *Vicia faba (V. faba)*

A summary of the array of effects obtained after treating *V. faba* root meristematic cells with the various plant extracts is presented in Table 4.

Abnormal metaphases were a major cytogenetic effect induced by the majority of the plant extracts following 2 hour treatment. These metaphases looked condensed or a clumpy mass of chromosomes (Fig. 9a), C-metaphases were also grouped in this category but were only observed in roots treated with the methanol extract of *M. salicifolia* (Fig. 9c).

From Table 4, Fig. 12(i) and 12 (iii), it can be seen

that the methanol extracts of *A. senegalensis*, *C. asiatica*, *M. africana*, *M. salicifolia* and the fresh sap of *A. graminicola* significantly enhanced the production of abnormal metaphases in a dose related fashion ($P < 0.05$) in roots fixed immediately after treatment.

Abnormal metaphases were also observed in roots allowed to recover for 20 hours but the results were not significant apart from those obtained from roots treated with the methanol extracts of *C. asiatica* and *M. salisfolia* [Fig. 12 (ii); Table 4].

Production of condensed metaphases has been reported in *V. faba* after treatment with 10 ppm of sulphite for 1 hour (Njagi, 1978). High doses of crude extract of *Cannabis* and its alkaloid papeverine hydrochloride, also produced clumpy metaphases in *A. cepa* (El-Bayoumi *et al.*, 1979; Malallah and Kabarity, 1982).

Condensed or clumpy metaphases may be due to chromosome stickiness resulting from certain physiological effects (Savage, 1976). Normally such stickiness is a transient phenomena which is found in cells after short exposure to irradiation. Those cells primarily affected, occur in late prophase and this is so because a few cells possessed bridges at prophase (Fig. 11). The C-metaphases observed in roots treated with *M. salicifolia* could be due to disruption of the spindle apparatus. In those roots, anaphases with disrupted spindle apparatus

were observed (Fig. 10)

Feulgen negative bands (Fig. 9d) observed on some of the C-metaphases could have been achromatic lesions (gaps) induced when cells are exposed at late prophase. The gaps were only observed in roots fixed immediately after treatment, confirming the fact that gaps are repairable and are never seen when cells are allowed to undergo a second division (Savage, 1976).

There might also be a possibility that such gaps could have been artifacts mainly associated with the aeration of the treatment solution. Neary and Evans (1958) found that the number of achromatic lesions (chromatid gaps) induced by x-irradiation decreased with a decrease in oxygen tension. Possibly in this case, they could have have been induced by low oxygen tension of the treatment solution.

Apart from the methanol extract of *M. lanceolata* all the other plant extracts produced chromosomal aberrations following two hours of treatment and 20 hours of recovery. However, only the methanol extracts of *A. senegalensis* *M. africana* and *M. salicifolia* seemed to have significantly enhanced the induction of chromosomal aberrations in a dose related fashion [$P < 0.05$; Fig 15(i)]. In recovery experiments, significant results ($P < 0.05$) were obtained only for *M. africana* and *M. salicifolia* [Fig. 15(ii)]. Lack of significance of some of these results

could possibly be due to sampling errors.

Chromosomal aberrations scored in the roots immediately after the treatment could be said to have occured in late G_2 (post-DNA synthesis) stage while those obtained in roots allowed to recover could have been induced in early G_2 or S phase. It can be deduced that these plant extracts contain clastogenic compounds of radiomimetic type which cause chromosome breakage at any stage of the cell cycle without the necessity to pass thr-ough S phase for the damage to be expressed, hence they cause non-delayed type of chromosomal aberrations. Chromo-somal aberrations in the form of fragments and bridges are reported in *V. faba* after treatment with various tropical medicinal plant extracts (Njagi, unpublished results) and similar observation were made in the root meristematic cells of *Allium cepa (A. cepa)* using an alkaloid of *Cannabis* (El-Bayoumi *et al.*, 1982).

Fragments result from double DNA-strand breaks (Revell, 1959). Anaphase fragments may form a variety of loops leading to bridges (Savage, 1976). Anaphase bridges may also be due to stickiness of the heterochro-omatic regions which are distributed at various loca-tions in *V. faba* chromosomes (Rowland, 1981). These heterochromatic regions separate later than the rest of the chromatid so that they are connected together by bridges at anaphase.

The Presence of micronuclei (MCN) in subsequent mitoses following treatment is an indicator of chromosome breakage which leads to loss of genetic material (Schmid, 1976). Such MCN were observed by Njagi (unpublished results) in *V. faba* root cells recovered for 23 hours in 4 tropical medicinal plant extracts, viz, *Catha edulis*, *Commiphora zimmermannii*, *Croton macrotachys* and *Croton megalocarpus* that contained bridges when fixed immediately after treatment. These results agree with those observed in the *V. faba* meristem cells after treatment with the fresh sap of *A. graminicola* after recovery (Fig. 16). There is a possibility that the other plant extracts might have produced MCN if the recovery period was increased since MCN may be produced even after one mitotic cycle. MCN in *A. cepa* were produced after long periods (48 hours) of treatment with *Cannabis* (Kabarity *et al.*, 1976) and that MCN persisted when *A. cepa* roots treated with pantapon hydrochloride (an alkaloid of*cannabis*) were recovered for 72 hours (Kabarity *et al.*, 1979).

Enlarged nuleoli was the main feature observed in cells treated with methanol extract of *M. lanceolata* (Fig. 18) and recovered for 20 hours but the results were not significant ($P > 0.05$, Fig. 19). These nucleoli could be attributed to swelling under unphysiological conditions (nucleolar oedema). Crude extracts of *Cannabis* were also

shown to produce enlarged nucleoli in root meristem cells of *A. cepa* (Malallah and Kabarity, 1982).

One limitation associated with root meristem cells in detection of medicinal plant extracts as genotoxic agents, is the apparent lack of synchrony. This could be responsible for the absence of statistical significance of some of the results obtained. Occurence of such asynchronous cells may present different sensivity to chemicals and aberrations might pass unobserved.

4.3. Conclusions

In this study, two short term test systems have been used to investigate whether medicinal plants contain genotoxic ingredients. The two tests measure different genetic end points: point mutations in *Salmonella*; and chromosomal aberrations in *V. faba*.

The *Salmonella* test was well suited in this investigation because it is sensitive and can recognise mutagenic impurities in complex mixtures the form in which human exposure occurs in real life. It also has an advantage in that metabolic activation system was added to the test compound taking into account effect of mammalian metabolism

It is, however, evident that bacterial tests do not pick up all classes of carcinogens with similar efficiency (Rinkus and Legator, 1975), thus chlorinated compounds do much less efficiently than polyaromatic hydrocarbons in the *Salmonella* test. For this reason, it is necessary to compensate for this by use of another test. Hence, the *V. faba* test for the induction of chromosomal aberrations was used, this test has advantages in that chromosomes of a higher organism with a similar morphology to that of man are used and that spontaneous aberrations in *V. faba* are very low (0.3%, Kihlman and Andersson,1984). It is known that

V. faba detects some mutagens which are not detected in mammalian cells such as maleic hydrazide (Kihlman and Andersson, 1984).

From the results obtained in this study, several plant extracts used in Kenyan traditional medicine are likely to contain mutagenic (genotoxic) ingredients. Some are activated by mammalian microsomal enzymes while certain others are deactivated to some extent. Further studies with purified extracts and other test systems are needed before recommendations regarding their continued use in traditional medicine can be made.

CHAPTER 5

REFERENCES AND APPENDICES

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5.2. Appendices

Appendix 1a - f

Mutagenic effect of medicinal plant extracts in the *Salmonella* tester strains.

One - factor analysis of variance (summary tables).

$P < 0.05$ (*)

$P < 0.01$ (**)

$P < 0.001$ (***)

df = degrees of freedom.

Appendix 1.

(a) *A. graminicola*

Tester strain	source of variance	Metabolic activation	Sum of squares	df	Mean sum of squares	F-ratio	Critical value of F, 0.05.
TA97a	Concentration	-S9	24990	5	4998	6.759**	3.11
		+S9	7456	5	1491	3.986*	3.11
	Error	-S9	8873	12	739.4		
		+S9	4489	12	374.1		
	Total	-S9	33860	17			
		+S9	11950	17			
TA98	Concentration	-S9	101.8	5	20.36	1.007 n.s.	3.11
		+S9	85.78	5	17.16	1.86 n.s.	3.11
	Error	-S9	242.7	12	20.22		
		+S9	110.7	12	9.222		
	Total	-S9	344.4	17			
		+S9	196.4	17			
TA100	Concentration	-S9	15250	5	3050	9.236***	3.11
		+S9	15680	5	3138	10.34***	3.11
	Error	-S9	3963	12	330.2		
		+S9	3644	12	303.7		
	Total	-S9	19210	17			
		+S9	19340	17			
TA102	Concentration	-S9	7174	5	1435	2.703 n.s.	3.11
		+S9	207100	5	41430	44.34***	3.11
	Error	-S9	6371	12	530.9		
		+S9	11210	12	934.2		
	Total	-S9	13550	17			
		+S9	218300	17			
TA104	Concentration	-S9	23810	5	4762	4.63*	3.11
		+S9	782600	5	156500	395.5***	3.11
	Error	-S9	12810	12	1067		
		+S9	4749	12	395.8		
	Total	-S9	36620	17			
		+S9	787400	17			

Appendix 1 continued.....

(b) *A. senegalensis*

Tester strain	Source of variance	Metabolic activation	sum of squares	df.	Mean sum of squares	F-ratio	Critical value of F, 0.05
TA97a	Concentration	-S9	109400	5	21890	6.812**	3.11
		+S9	449800	5	89970	60.18***	3.11
	Error	-S9	38560	12	3213		
		+S9	17940	12	1495		
	Total	-S9	148000	17			
		+S9	467800	17			
TA98a	Concentration	-S9	245900	5	49170	98.36***	3.11
		+S9	337000	5	67400	359.1***	3.11
	Error	-S9	5999	12	4.999		
		+S9	2252	12	187.7		
	Total	-S9	252000	17			
		+S9	339200	17			
TA100	Concentration	-S9	720500	5	144100	47.66***	3.11
		+S9	375000	5	75000	162***	3.11
	Error	-S9	36280	12	3023		
		+S9	0.5555	12	462.9		
	Total	-S9	756800	17			
		+S9		17			
TA102	Concentration	-S9	191900	5	38380	320.4***	3.11
		+S9	207300	5	41450	183.4***	3.11
	Error	-S9	1437	12	119.8		
		+S9	2712	12	226		
	Total	-S9	193300	17			
		+S9	210000	17			
TA104	Concentration	-S9	324600	5	64930	159***	3.11
		+S9	636100	5	127200	246.9***	3.11
	Error	-S9	4899	12	408.2		
		+S9	6183	12	515.3		
	Total	-S9	329500	17			
		+S9	642300	17			

Appendix 1 continued.....

(c) *C. asiatica*

Tester strain	Source of variation	Metabolic activation	Sum of squares	df.	Mean sum of squares	F - ratio	Critical value of F,0.05
TA97a	Concentration	-S9	3384	5	676.7	2.645 n.s	3.11
		+S9	14660	5	2931	23.13***	3.11
	Error	-S9	3070	12	255.8		
		+S9	1521	12	126.7		
	Total	-S9	6454	17			
		+S9	16180	17			
TA98	Concentration	-S9	112300	5	22460	227.7***	3.11
		+S9	168600	5	33730	219.7***	3.11
	Error	-S9	1183	12	98.61		
		+S9	1843	12	153.6		
	Total	-S9	113500	17			
		+S9	170500	17			
TA100	Concentration	-S9	68630	5	13730	43.24***	3.11
		+S9	292700	5	58530	342.7***	3.11
	Error	-S9	3809	12	317.4		
		+S9	2049	12	170.8		
	Total	-S9	72440	17			
		+S9	294700	17			
TA102	Concentration	-S9	147000	5	29410	81.36***	3.11
		+S9	670300	5	135300	127.5***	3.11
	Error	-S9	4337	12	361.4		
		+S9	12730	12	1061		
	Total	-S9	151400	17			
		+S9	689000	17			
TA104	Concentration	-S9	324600	5	64930	159***	3.11
		+S9	636100	5	127200	246.9***	3.11
	Error	-S9	4899	12	408.2		
		+S9	6183	12	515.3		
	Total	-S9	329500	17			
		+S9	642300	17			

Appendix 1 continued.....

(d) *M. lanceolata*

Tester strain	Source of variance	Metabolic activation	Sum of squares	df.	Mean sum of squares	F-ratio	Critical value of F,0.05
TA97a	Concentration	-S9	25810	5	5162	4.568**	3.11
		+S9	13410	5	2682	4.206**	3.11
	Error	-S9	13560	12	1130		
		+S9	7653	12	4.206		
	Total	-S9	39370	17			
		+S9	21070	17			
TA98	Concentration	-S9	319.8	5	63.97	2.374 n.s.	3.11
		+S9	1534	5	306.8	2.932 n.s.	3.11
	Error	-S9	320.7	12	26.72		
		+S9	1255	12	104.6		
	Total	-S9	640.5	17			
		+S9	2789	17			
TA100	Concentration	-S9	3693	5	738.6	0.5563 n.s.	3.11
		+S9	261500	5	52310	0.9454 n.s.	3.11
	Error	-S9	15930	12	1328		
		+S9	663900	12	55330		
	Total	-S9	19630	17			
		+S9	925400	17			
TA102	Concentration	-S9	89820	5	17960	14.63***	3.11
		+S9	271900	5	54380	127.2***	3.11
	Error	-S9	14740	12	1228		
		+S9	5129	12	4274		
	Total	-S9	104600	17			
		+S9	277000	17			
TA104	Concentration	-S9	150700	5	30150	52.48***	3.11
		+S9	104500	5	20910	7.392**	3.11
	Error	-S9	6893	12	574.4		
		+S9	33940	12	2829		
	Total	-S9	157600	17			
		+S9	13850	17			

Appendix 1 continued.....

(e) *M. africana*

Tester strain	Source of variance	Metabolic activation	Sum of squares	df.	Mean sum of squares	F-ratio	Critical value of F, 0.05
TA97a	Concentration	-S9	11060	5	2212	7.101**	3.11
		+S9	204000	5	40800	52.51***	3.11
	Error	-S9	3739	12	311.6		
		+S9	9325	12	777.1		
	Total	-S9	14800	17			
		+S9	213300	17			
TA98	Concentration	-S9	125.3	5	25.07	0.7083 n.s.	3.11
		+S9	112100	5	22430	4.765*	3.11
	Error	-S9	424.7	12	35.39		
		+S9	56480	12	4707		
	Total	-S9	550	17			
		+S9	168600	17			
TA100	Concentration	-S9	258.7	5	51.73	2.83 n.s.	3.11
		+S9	5336	5	1071	5.081**	3.11
	Error	-S9	219.3	12	1828		
		+S9	2530	12	210.8		
	Total	-S9	478	17			
		+S9	7886	17			
TA102	Concentration	-S9	12150	5	2431	3.779*	3.11
		+S9	4912	5	982	1.944 n.s.	3.11
	Error	-S9	15000	12	1250		
		+S9	3120	12	260		
	Total	-S9	27160	17			
		+S9	8033	17			
TA104	Concentration	-S9	20270	5	4053	12.65***	3.11
		+S9	445400	5	89090	514***	3.11
	Error	-S9	3844	12	320.3		
		+S9	2077	12	173.1		
	Total	-S9	24110	17			
		+S9	447500	17			

Appendix 1 continued.....

(f)*M. salicifolia*

Tester strain	source of variance	Metabolic activation	Sum of squares	df.	Mean sum of squares	F-ratio	Critical value of F, 0.05
TA97a	Concentration	-S9	159400	5	7561	6.744**	3.11
		+S9	37800	5	31880	29.15***	3.11
	Error	-S9	13120	12	1121.		
		+S9	13450	12	1093		
	Total	-S9	51260	17			
		+S9	172500	17			
TA98	Concentration	-S9	64	5	12.8	0.51n.s.	3.11
		+S9	192100	5	38420	32.79***	3.11
	Error	-S9	304	12	25.33		
		+S9	14060	12	1172		
	Total	-S9	368	17			
		+S9	206200	17			
TA100	Concentration	-S9	6071	5	1214	6.877**	3.11
		+S9	133300	5	26670	29.98***	3.11
	Error	-S9	2113	12	176.1		
		+S9	10680	12	889.7		
	Total	-S9	8184	17			
		+S9	144000	17			
TA102	Concentration	-S9	4262	5	852.4	1.19n.s.	3.11
		+S9		5	540	2.30n.s.	3.11
	Error	-S9	8572	12	714.3		
		+S9		12	234		
	Total	-S9	12830	17			
		+S9		17			
TA104	Concentration	-S9	282300	5	56450	12.37***	3.11
		+S9	620700	5	124100	276.5***	3.11
	Error	-S9	54770	12	4564		
		+S9	5389	12	449.1		
	Total	-S9	337000	17			
		+S9	626100	17			

Appendix 2.

Mutagenic effect of the medicinal plant extracts in the *Salmonella* tester strains.
Summary tables of means at various dose levels and their differences in a t - test.

LSD = Least significant difference between any 2 concentrations which would be significant in a Students t - test (P = 0.05).

-S9 = Without metabolic activation.

+S9 = With metabolic activation.

(a) *A. graminicola.*

Strains	S9	LSD	Dose(µl/plate)					
			0	62.5	125	250	500	1000
97	-S9	34.2	88.7a	166b	159b	168bc	200bc	200c
	+S9	24.3	99.7a	94.7a	107a	109a	134b	152b
100	-S9	26.3	125a	101a	153b	170b	169b	168b
	+S9	4.9	99a	106a	135b	152b	164bc	181c
102	+S9	38.5	259a	458b	561c	567c	522cd	543d
104	-S9	43.7	223a	243a	221a	261ab	290bc	309bc
	+S9	24.7	350a	509b	563c	667d	837e	979f

Means followed by a common letter are not significantly different.

(b) *A. senegalensis*

Strains	S9	LSD	Dose(µg/plate)					
			0	62.5	125	250	500	1000
97	-S9	71.3	151a	351bc	321bc	341bd	401d	317b
	+S9	48.7	146a	167a	258b	322c	356c	623d
98	-S9	28.1	28a	117b	164c	143bc	263d	396e
	+S9	17.2	42a	142b	356c	416d	385e	316f
100	-S9	69.2	128a	136a	257b	644c	529d	489d
	+S9	27.1	166a	152a	274b	45ce	489d	481de
102	-S9	13.8	205a	312b	399c	421d	477e	513f
	+S9	18.9	228a	341b	451c	480d	515e	532e
104	+S9	25.4	341a	414b	604c	620c	639cd	725d
	+S9	28.6	294a	342b	541c	642d	646d	843e

Means followed by a common letter are not significantly different.

Appendix 2 continued.....

(c) *C. asiatica*

Strains	S9	LSD	Dose (µg/plate)					
			0	62.5	125	250	500	1000
97	+S9	14.2	163a	203b	245c	249c	223d	216b
98	-S9	12.5	29.7a	107b	130c	181d	245e	255e
	+S9	15.6	29a	168b	198c	210c	302d	325e
100	-S9	22.4	152a	225b	250c	315d	324d	314de
	+S9	16.4	121a	237b	311c	405d	434e	499f
102	-S9	23.9	244a	332b	402c	454d	476d	506e
	+S9	41	329a	396b	634c	825d	809d	734e
104	-S9	54.4	341a	380ab	364ac	467d	546e	526e
	+S9	94.2	382a	405ab	564c	472ac	617d	536d

Means followed by a common letter are not significantly different.

(d) *M. lanceolata*

Strains	S9	LSD	Dose (µg/plate)					
			0	62.5	125	250	500	1000
97	-S9	42.3	115a	129ab	186c	230d	173c	159bc
	+S9	31.8	131a	209b	186b	216b	179bc	179c
102	-S9	44.1	147a	171a	234b	308cd	338d	290c
	+S9	26	145a	221b	288cd	303d	339c	543f
104	-S9	30.2	275a	365b	437c	493d	489d	552e
	+S9	66.9	317a	317b	443c	353d	421c	529d

Means followed by a common letter are not significantly different.

(e) *M. africana*

Strains	S9	LSD	Dose (µg/plate)					
			0	62.5	125	250	500	1000
97	-S9	22.2	90.7a	93.7a	128ab	151b	151c	109a
	+S9	35.1	135a	153a	147a	213b	321c	424d
98	+S9	86.4	37.3ab	953bc	176c	253c	253c	192c
100	+S9	31.8	115a	123ab	122ab	132ac	159c	103a
102	+S9	20.3	329a	317a	318a	335a	322a	365b
104	-S9	22.5	310a	328a	407b	369c	366c	321a
	+S9	16.6	321a	350b	504c	595d	769e	632f

Means followed by a common letter are not significantly different.

Appendix 2 continued.....

(f) *M. salicifolia*

Strains	S9	LSD	Dose (µg/plate)					
			0	62.5	125	250	500	1000
TA97a	-S9	42.1	239a	312b	360c	319bc	247a	245a
	+S9	41.6	149a	373b	379b	427c	397bc	410bc
TA98	+S9	43.1	35.3a	263b	360c	319cd	281bd	245b
TA100	-S9	16.7	91.3a	129b	129bc	102a	130c	145c
	+S9	43.1	221a	426b	443b	467b	463b	448b
TA104	-S9	85	411a	396a	433ab	727c	630b	445d
	+S9	26.7	397a	442b	459b	853c	831c	521d

Means followed by a common letter are not significantly different.

Appendix 3. Regression Anova (Summary tables) for the chromosomal aberrations caused by medicinal plant extracts in *V. faba* root meristem cells

Levels of significance:

$P < 0.05$ (*)
$P < 0.01$ (**)
$P > 0.05$ (n.s.)

(a) Roots fixed immediately after treatment.

(i) *A. graminicola*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	210.1	1	210.1	2.483 n.s.	10.1
residual	253.9	3	84.62		
Total	464	4			

(ii) *A. senegalesis*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	40.5	1	40.5	0.529 n.s.	10.1
residual	229.5	3	76.5		
Total	270	4			

(iii) *C. asiatica*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	1540	1	1540	4.46 n.s.	10.1
residual	1036	3	345.3		
Total	2576	4			

Appendix 3 continued.....

(iv) *M. africana*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	3081	1	3081	64.7**	10.1
residual	142.9	3	47.62		
Total	3224	4			

(v) *M. salicifolia*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	351.1	1	351.1	16.75*	10.1
residual	62.87	3	20.96		
Total	414	4			

Appendix 3 continued....

b. Roots allowed to recover for 20 hours.

(i) *A. senegalensis*

Source of variation	sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	2380	1	2380	43.15**	10.1
residual	165.5	3	55.17		
Total	2546	4			

(ii) *C. asiatica*

Source of variatiom	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	4.5	1	4.5	1.421 n.s.	10.1
residual	9.5	3	3.167		
Total	14	4			

(iii) *M. africana*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	512	1	512	20.76*	10.1
residual	74	3	24.67		
Total	586	4			

(iv) *M. salicifolia*

Source of variation	Sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	3321	1	3321	35.22**	10.1
residual	282.9	3	94.29		
Total	3603.9	4			

Appendix 4. Regression Anova (Summary tables for abnormal metaphases caused by medicinal plant extracts in *V. faba* root meristem cells.

Levels of significance:

P < 0.05 (*)
P > 0.05 (n.s)

(a) Roots fixed immediately after treatment.

(i) *A. graminicola*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	325.1	1	325.1	19.96*	10.1
residual	48.87	3	16		
Total	374	4			

(ii) *A. senegalensis.*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	861.1	1	861.1	27.23*	10.1
residual	94.87	3	31.62		
Total	956	4			

(iii) *C. asiatica*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	420.5	1	420.5	10.92*	10.1
residual	115.5	3	38		
Total	536	4			

Appendix 4 continued.....

(iv) *M. africana*

Source of variation	sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	1300	1	1300	23.57*	10.1
residual	165.5	3	55.17		
Total	1466	4			

(v) *M. salicifolia*.

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	4656	1	4656	11.9*	10.1
residual	1174	3	391.3		
Total	5830	4			

(b) Roots allowed to recover for 20 hours.

(i) *A. graminicola*

Source of variation	sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	1985	1	1985	5.828 n.s.	10.1
residual	1021	3	540.5		
Total	3006	4			

(ii) *C. asiatica.*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	465.1	1	465.1	28.55*	10.1
residual	48.87	3	16.29		
Total	514	4			

Appendix 4 continued.....

(iii) *M. africana*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	153.1	1	153.1	8.688 n.s.	10.1
residual	52.87	3	17.62		
Total	206	4			

(iv) *M. salicifolia*

Source of variation	Sum of squares	df	Mean squares	F-ratio	Critical value of F, 0.05
Line	5356	1	5356	29.33*	10.1
residual	547	3	182.6		
Total	5904	4			

Appendix 5. Regression anova (Summary table) for size of nucleoli in root meristems of *V. faba* treated with the methanol extract of *M. lanceolata*.

Levels of significance:
P > 0.05 (n.s.)

Source of variation	sum of squares	df	Mean square	F-ratio	Critical value of F, 0.05
Line	5.515x10	1	5.515x10	5.564 n.s.	10.1
residual	2.973x10	3	9.911x10		
Total	8.44x10	4			