GENOTOXICITY STUDIES ON *LIZA PARSIA* AND *MUGIL CEPHALUS*

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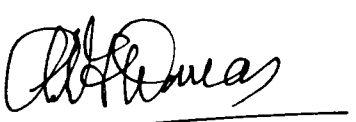
C E R T I F I C A T E

This is to certify that thesis entitled "Genotoxicity studies on Liza parsia and Mugil cephalus" is the bonafide record of the research work carried out by Shri K.Madhu under my guidance and supervision at the Post Graduate Programme in Mariculture, Central Marine Fisheries Research Institute (CMFRI), and that no part thereof has been presented for the award of any other degree.



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C O N T E N T S

P R E F A C E

Mullets are mainly distributed in tropical and subtropical waters around the world and a few of them occur in temperate zones. Liza parsia and Mugil cephalus are distributed in west and east coast of India. Adults and young ones are available in the sea and juveniles are found in low saline area (brackishwater area). These two species are commercially important, of which L.parsia form a good fishery in the estuaries of India (Jhingran, 1991). Both species are considered to be model fish for genotoxicity studies, because they have small diploid number, large size chromosome, same type of chromosome, available in polluted and non-polluted areas, easily acclimatised in laboratory condition, etc. There are various reports about pollution from industries which are situated on the banks of Cochin backwater, due to effluents discharged into this estuarine system. So many authentic studies were conducted about pollution in Cochin backwater. The pollution may affect the fish ecosystem which may also affect the genome level of fish.

Cytogenetics is the study of chromosome morphology and the behaviour of chromosome during meiosis and mitosis. Karyomorphological studies of species can give the basic information regarding the chromosome number, size and shape, which is a prerequisite for any chromosomal change study. Fishes have been subjected to constant effects of various kinds of pollutants in nature. This has resulted in several mutagenic changes which attracted the attention of recent

investigators. It is well known that the pollutants like pesticides, oil, factory effluents, sewage effluents, metals, etc. can affect the fishes in different ways.

In India, several observation were made to examine the aquatic pollution. But the studies on pollution effect on genome level were scanty. The chromosome aberrations and micronuclei were studied by a few workers only. Moreover, the standard protocol based on mammalian systems need to be standardised for fish.

Various type of pesticides and synthetic chemicals are widely used in agriculture. Eventhough application of pesticides is essential for crop protection, it is harmful to other organisms as they ultimately reach the aquatic system and finally adversely affect the aquatic organisms, including fish. The concentration of the pesticide in the aquatic system should be monitored, because it will affect the biochemical and chromosomal changes in the aquatic animals. The long term effect on the tissue of organisms will result in damages inside the organisms. Organophosphorous, organochlorine, thiocyanate, carbomate, etc. are important pesticides. In Kerala, organophosphorous pesticides are extensively used.

In the present study following aspects were covered.

1. Standardisation of methodology for in vivo chromosome preparation. The five methods (Mc Phail and Jones, 1966; Chen and Ebeling,

1968; Denton and Howell, 1969; Kligerman and Bloom, 1977 and Reddy and John, 1986) were used to develop methodology for candidate species.

2. Screening of suitable species as cytogenetic model to study the genotoxic effects. Lates calcarifer, Scatophagus argus, Liza parsia and Mugil cephalus were screened for this study.

3. L.parsia and M.cephalus were selected for the present study.

4. Karyotype of two candidate species were prepared.

5. Determined the diploid number of chromosomes in the two species.

6. Six type methods (Schemid, 1976; Salamone et al., 1980; Hooftman and de Raat, 1982; Walton et al., 1984; Manna and Sadhukhan, 1986 and Hose et al., 1987) were used to develop methodology for micronuclei studies.

7. Base line chromosome aberrations and micronuclei formation of these two species were studied.

8. Five types of pesticides (methyl parathion, phosphamidon, dichlorvos, monocrotophos , malathion) which are extensively used in agriculture in Kerala, were used to study the induced chromosome aberration and micronuclei formation.

9. The result tabulation and discussion were carried out in separate sections in the thesis.

I N T R O D U C T I O N

In modern civilization, aquatic sources are enormously used as the dumping ground for man made waste which are transformed into most toxic compound in aquatic systems. The aquatic toxicologist, however, revealed the great danger of its effects on xenobiotics in structure and function of aquatic ecosystem. Modern geneticist proposed the link between pollution and genome of aquatic animals. Genetic toxicology, therefore, emerged as a new field of genetics, especially fish genetics. Several cytogenetic assays were developed by modern genetic toxicologist. The genetic toxicology was recognized as a separate branch around 1969 after the Environmental Mutagen Society was established.

The environmental mutagens have been classified into three, such as, living, physical and chemical mutagen (Manna, 1982, 1983). The consequence of these pollutions on fin fish and shell fish have been studied by toxicologist. The exploratory research in this field has identified it as an important environmental problem, all over the world. The effectiveness of these chemicals on hereditary components of living organisms were categorised in genetic toxicology. Brusick (1980) reported that even if many toxic substance damage the genetic material in nonspecific manner, the effect of these agents are highly specific on nucleic acid. Hence these are capable

of producing harmful effect at sublethal level.

Mutations are caused by many factors in environment and these lead to chromosomal structural changes. This may also result in changes in DNA and gene level. It is not possible to eliminate all mutagens and carcinogens in environment. It is, therefore, important to know the relative strength of mutagenic potency of environmental chemicals. Such information is vital for risk assessment and utility for the regulation of chemicals. Assessment of the risk posed by a chemical, generally, is based not only on whether the chemicals appears to constitute a health hazard but also on the actual magnitude of exposure to the organisms. Mutation research plays a very important role in predicting the toxicity of materials and judging their hazard, and impact on the population and the environment.

Various short term test (STTs) have been developed over the past 20 years to evaluate the mutagens which employ a variety of targets (bacteria, mammalian cell lines, insects, plants, fishes, etc.) with different end points like, chromosomal effect, micronuclei formation, DNA damage, gene mutation, etc. as a result of the recent developments in the field of genetic toxicity.

Many components even at sublethal level are genetically active and can cause DNA damage, the consequence of which get persistence

in long term. These mutagenic compounds may leads to changes in the gene pool with unpredictable population genetic consequences.

Among the various models used for the genotoxicity studies, fishes appear to be the best for more than one reason (Manna, 1987) viz. ready availability, suitability for karyotype, ability to withstand experimental stress and availability in polluted and non-polluted areas. Oreochromis mossambica has been advocated to be an ideal model for this type of study (Manna, 1986). Salmo gairdneri, Umbra limi, Notobranchius rachowi, Oryzias latepes have been used as model fishes for genotoxicity studies by different workers (Manna, 1984).

The chromosome aberration test has been considered as an authentic one in assessing the mutagenic potentiality of physical and chemical agents (Manna, 1989). In vivo and in vitro methods have been followed for preparing chromosome of fishes. Gill, kidney, etc. for somatic chromosomes and testes for germinal chromosomes have been commonly used for fish chromosome studies (karyotyping, banding, chromosome aberration, etc.). In making the good spread of metaphase the colchicine, hypotonic, acetic-alcohol, air/flame dry, Giemsa staining scheduled were commonly followed.

Micronucleus test (MNT) is another better method to evaluate genotoxic effects which is considered as quicker authentic screening test (Manna and Biswas, 1986). Tilapia and Indian major carps were used as

model organism to study the various mutagens in smear of peripheral blood as well as in cells of kidney and gills (Manna, 1983; Manna and Biswas, 1986 and Sadhukhan and Manna, 1986). Air dried blood smears fixed in methanol, followed by May-Grunwald and Giemsa staining have been commonly used in micronuclei study.

Based on the previous investigations, the present study was conducted to observe the genotoxic effect of certain organophosphorous pesticides on L.parsia and M.cephalus. The gill tissue was used to study the diploid number, karyotype and chromosome aberration of tested animals while peripheral blood was taken for micronuclei study. Comparisons of karyotypes, effect of genotoxic agents, chromosome aberrations, micronuclei formations, behavioural changes and significant level of effects of these pesticides were also carried out between the candidate species.

R E V I E W O F L I T E R A T U R E

1. CHROMOSOME STUDIES

The chromosome morphology and its behaviour during meiosis and mitosis were studied in cytogenetics. The fish genetics is in an infant stage as compared with mammalian genetics. Fish cytogenetics began as a separate discipline, a couple of decades before. Although about 20,000 fish species were reported in the world, the information about Karyomorphology of fish is available from approximately 1700 species which contain about 600 genera, 155 families and 36 orders (Rishi, 1989). Therefore, most of the fishes are Karyomorphologically unknown.

Chromosome numbers (2n) in fishes ranges from 12 to 239±7 (diploid number 12 in Gonostoma bathyphylum and 239±7 in Acipenser naccarii, Rishi, 1989). The peak at 2n=48 showed in majority of families. Analysis of karyotypes, diploid numbers, and its behaviour and arrangement have helped the taxonomist as tools to study the interrelationship of different group of animals. However, meristic counts and morphometric analysis have been considered as tool by taxonomist which may not be ubiquitous. Hence, the chromosomal information of the species are essential to modern taxonomist. Besides, the chromosome information gives direction to assess the environmental mutagen. The review attempted here has been made only as an outline of past studies in fish cytogenetics.

The work on fish chromosome began with study of Schwarz in 1887 who tried to count the fish chromosome accurately (Savardson, 1945). But, Makino (1951) reported that first attempts began a century back on Agnathan, Myxine glutinosa by Retziat (1890) on shark, Pristiurus melanostomus by Katschenko (1890) and on Salmo fario by Bohm (1891). But, the revolutionised cytogenetics was initiated by the study of Tijo and Levan (1956). Further developments in fish chromosome study took place when the colchicine was used for pretreatment of embryological material (Simon, 1963; Simon and Dollar, 1963; Swarup, 1959); sectioning of testis (Nogusa, 1960); corneal and conjunctival epithelium (Sick et al., 1962; Drewry and Howell, 1964); in squash preparations of testes, gill and haematopoetic tissue (Roberts, 1964; Ohno et al., 1965; Mc Phail and Jones, 1966; Davisson, 1972); in vitro studies (Roberts, 1966, 1967); gill epithelium (Chen and Ebeling, 1968; Setzer 1970; Campos, 1972); Scale epithelium (Denton and Howell, 1969). Levan et al. (1964) suggested the standardised nomenclature for centromeric position of chromosomes. Ojima and Hitotsumachi (1967); Manna and Prasad (1968, 1971, 1973, a,b,c, 1974a,b, 1976) reported air and flame drying methods with the aid of mitotic inhibitor like colchicine or colcemid, and hypotonic treatment of the cells and subsequent preparation for metaphase. Mayers and Roberts (1969) studied the chromosomal homogenicity of five populations of Alewites, Alosa pseudoherengus. Howell (1972) reported somatic chromosome of the black ghost knife fish, Apteronotus albiforms. Davisson (1972) studied the karyotype of teleost family Esocidae. Evolutionary implication of five species

in this family were discussed in this report. Miller (1972) studied the classification of the trouts of Arizona with description of new species, Salmo apache is distinguished by a number of characters such as life colour, spotting body proportions, number of scale , vertebra and pyloric caeca, occasional presence of basibranchial teeth and by its Karyotype (2n=56). Fitzsimons (1972) also studied two genera of goodied fishes (Cyprinodontiformes and Osteichthyes). These two genera of the goodied fishes were evaluated with conventional information on morphological features and new information on karyotypes, courtship behaviour, hybridization experiment and discrimination test. Meredith (1969); Evan et al. (1972); Stock et al. (1972); Blaxhall (1975); Chen (1970) have given systematic methods for chromosome preparation. These methods were modified as an easy and rapid chromosome preparation from solid tissue of fishes by Kligerman and Bloom (1977). Ojima et al. (1973) reported the Karyotype of 15 Japanese cyprinids. Chiarelli and Capanna (1973), Denton (1973), Ohno (1970, 1974) reported that due to technical difficulties of early workers, results of several reports of chromosome number and morphology were considered incorrect. Cataudella and Capanna (1973), Cataudella et al. (1974); Le Grande and Fitzsimons (1975) studied the chromosome of mullet. The karyology of six species of Lousiana flat fishes, Paralichthys lethostigma, Etropus crossotus, Citharicthys spilopterys, Trinectes maculatus, Achirus lineatus and Symphurus plagiusa were studied by Le Grande (1975). Labat et al.(1967), Heckman and Brubaker(1970),Ojima et al.(1970),Chen(1970),

Heckman et at.(1971) Barker (1972), Etlinger et al. (1976) and Al-Sabti (1985 c) described that improvements in protocol were possible by developing method for in vitro study. Merriless (1975) observed that the chromosome complements of Galaxias maculatus from New Zealand differ from that of Australian and Chilean specimens.

Gjedrem et al. (1977) reported the chromosome number of salmonids and its hybrids. Karyotype of fishes and technique of chromosome preparation were elaborately described by Gold (1979), that only 20,000-23,000 living species of fishes were taxonomically described. Of these, chromosome number is known only for about 650-700 species and complete karyotype has been made in about 500 species. The main obstacle of early cytologist was the numerous technical difficulties. Vasil'yew(1980) published the check list of diploid number of fish species.

Ojima and Kurishita (1982) reported the in vivo and in vitro method for fish chromosome preparation. Hartley and Horne (1983), Blaxhall(1983) reported the methodology for in vitro chromosome preparation in fishes. The in vitro chromosome studies on the salmonids (rainbow trout, Salmo gairdneri, brown trout, S. trutta and mirable trout, S. marmoratus) from Yugoslavian waters have been studied by Al-Sabti (1984, 1985 b,c). Amemiya et al.(1984) studied the simple and reliable short term monolayer cell culture technique for chromosome preparation from small specimens of North American

Cyprinids fishes. The occurrences of multiple sex chromosome in Gobionellus shufeldti were reported from mitotic and meiotic chromosome spread by Pezold (1984) and in the annual killi fish, Notobranchius guntheri by Ewulonu et al.(1985). The developments in fish cell culture in biomedical research were reviewed by Hightower and Renfero (1988). Arefev (1989) used the colchicine bath technique to study the karyotype of two Black Sea mullet. Cucchi and Baruffaldi (1989) observed the effects of phenyl hydrazine in in vivo chromosome preparation. This chemical will induce the haematopoetic tissue to actively proliferate the cells which will help to yield higher number of metaphase. This techniques cannot be applied to small specimens. To over come such problem, a new method has been set up using cobalt chloride by Cucchi and Baruffaldi(1990). Rab and Jankun (1990) reviewed the chromosome studies in coregonic fishes whereas karyological information of Coregoninae were given by Frolov (1990) and Makoedov (1990). Short term cell culture technique for obtaining chromosome in marine fishes like gilthead seabream, Sparus auratus and cardinal fish, Apgon imberbis, and freshwater fishes like northern pike, Esox lucius, gold fish, Carassius auratus were studied by Alwarez et al. (1991). The inter and intra populational karyotypic variation ie, 2n=34 in southern race and 2n=38-40 in northern race in Chile, of Liolaemus monticola were studied by Lanbrot (1991). Chavez Justo et al.(1991) reported the diploid chromosome number (2n = 118) of fresh water prawn, Macrobrachium rosenbergii. The chromosome configuration revealed that 45 meta and submetacentric and 14 subtelo and acrocentric pairs.

The chromosomes of the kidney and gill cells of Pangatius species were demonstrated according to the direct routine air drying method with giemsa staining, and Levan's classification was followed to study the karyotype (Suzuki and Takeda, 1991). Joradao et al. (1992) reported the mitotic chromosome, the Nucleolar Organizer Regions (NORs) and distribution pattern of constitutive heterochromatin of the mullet Mugil platanus. Gomes et al.(1992) studied the diploid number of chromosome of cathorops, a marine cat fish which has the diploid number of 54 chromosomes. Diploid number (2n=70) in Scyllarus arcutus and diploid number(2n=126) in S.tatus from mitotic and meiotic chromosome were reported by Salvadori et al.(1992).The chromosome and DNA content of eight species of Japan,China to South east Asian cobitid fishes were reportedby Suzuki (1992). The premature chromosome condensation in kidney cells of Eigenmania virescens were reported by Foresti et al. (1992). Gajardo (1992) observed the karyotype of Basilichthys mocrolepidotus and B.australis and discussed the evolutionary significance of chromosome information. Karyotype of sandlance species, Gymnammodytes cicerelus, C-banding and Nucleolar organizer Region (NOR) by silver staining were studied by Amores et al. (1993). Valcarcel et al. (1993) investigated the karyological characteristic of south American catfish, Rhamdia sapo. Mair (1993) reported the technique, problems and prospects of chromosome set manipulation in tilapia. The advance in fish cell engineering in China were described by Lu and Chen (1993). Ieyama (1993) studied the karyotype of Dentalium octanogulatum and Fissidentalium pictodentalium vernedei. A new technique have been evolved for

chromosome preparation from fish specimens which was reported by Foresti et al. (1993). Gomez et al. (1993) observed the comparative karyotypic analysis in both sex of European eel, Anquilla anguilla. Rebelo porto et al. (1993) have conducted the cytogenetic studies in seven Hemiodidae fishes from the central Amazon. All fishes have similar macrostructure with 2n=54 and FN - 108. G-banding pattern of Chines crucian carp from cultured fin cells were reported by Takai and Ojima (1993). Mao Lianju et al. (1993) conducted studies the karyotype of Ernogrammus hexagrammus. In this study all telocentric chromosome were reported from the spleen tissue of E.hexagrammus by the colchicine hypotonic air drying Giemsa staining method. Diploid number of chromosome (2n = 48) of this species was also reported. The diploid number 2n=50 and arm number 82, 84 and 88 of three cyprinid species (Garra dembensis G.najuebsus and G. guandnmaculate were studied by Krysanov and Golubstov (1993).

Chromosomal NOR karyotypes and genome size variation among squawfishes of the genus Ptychocheilus were reported by Gold and Li (1994). The diploid number (2n=36) of chromosome in fresh water snail, Semisulcospira reiniana were reported by Nakano et al. (1994). Di Shaojie and Knowled (1994) reported the confirmation of diploid number (2n=46) of chromosome in dab, Limanda limanda. The heteromorphic sex chromosomes in the gobid, Eleotris pisonis were reported by Uribe-Alcocer (1994). The karyotypic and taxonomic

diversity within the sub order Notothenoidei were reported by Priorodina (1994). Klinkhardt (1994) reported the chromosome morphologies and number showed variability among the species of Gadidae and were estimated as 2n=26 in Eleginus navaga ,2n=46 in Gadus morha marisalbi and 2n=48 in Lota lota.

Pioneering contribution in fish cytogenetics in India were given by Sharma et al.(1960). Chromosome of twentytwo freshwater species were studied by Nayyar (1962, 1964, 1965, 1966) and five species were studied by Kaur and Srivastava (1965). The diploid number (2n=54) chromosome in spermatagonial tissue of Labeo goneius and L.dero (Cyprinids) were reported by Nayyar (1962) and diploid number (2n=48) chromosome of Notopterus chitala and N.notopterus were reported by Nayyar (1965). Natarajan and Subramanyan (1968) reported the chromosome number of Tilapia mossambica. Kidney tissue was used for chromosome preparation in Channa punctatus by Manna and Prasad (1968) who have made enough contribution in the field of fish cytogenetics in India. Srivastava and Das (1969) studied the somatic chromosome prepared from colchicine treated tissues by air drying method of Ford and Hamerton. Karyotype of the estuarine fish, Bleopthalmus boddaeri (Pallas) were studied by Subramanyam (1969). Karyological studies on somatic and germinal tissues of Channa sp, Anabas testudineus and Puntius spp. were conducted by Manna and Prasad (1973 a, b, c). Rishi(1973) observed the karyotype of eighteen marine fishes. The meiotic chromosome of the Indian teleosts were conducted by Das and Srivastava (1973). Two forms of Mystus vittatus were

treated as two distinct species based on chromosomal evidence (Manna and Prasad, 1974 b). Somatic chromosome of two hybrid carps were studied by Manna and Khuda Bukhsh (1974). Natarajan and Subramanyam (1974) conducted karyotype study of teleosts from Porto Nova waters. They claimed that the diploid number of Eteroplus suretensis was 48 and E. maculatus was 46. Somatic and meiotic chromosomes in Trichogaster fasiatus were studies by Rishi (1975). Khuda Bukhsh and Manna (1976 a) studied the chromosome of two species of Indian mullets. Karyomorphology and morphometric measurements of mitotic chromosomes in males of Mugil parsia, M.corsula and spermatocytic chromosome in M.parsia, and comparison of karyotype of these species were discussed in this study. Analysis of chromosome in two Indian hybrid carps were also studied by Khuda Bukhsh and Manna (1976 b). Rishi (1976 a,b) reported mitotic and meiotic chromosome with indications of male heterogamety in Callichrous bimaculatus. Rishi and Gaur (1976) had given chromosomal evidence for female heterogamety in jet black molly, Molliensia sphenops. Chromosome studies in Tilapia mossambica and Notopterus notopterus were conducted by Prasad and Manna (1976). Gill epithelium, kidney and spleen tissue of Heteropneustes fossilis, Clarius batrachus, Colisa latius, Danio (Brachydanio) rerio were used to study the karyotype of these four fishes (Rishi, 1976). Manna and Khuda Bukhsh (1977) prepared a check list of diploid number in cyprinid species and cytologically evaluated the cyprinids. Manna and Khuda Bukhsh

(1977 and 1978) studied morphometrical analysis of chromosome complements of number of freshwater species.

Sharma and Agarwal (1978) studied the somatic chromosomes from kidney and spleen of Channa punctatus (2n=32) and C gachua (2n=78). Giemsa banding in chromosome of Channa punctatus, Colisa fasciata, Mystus tengra., Puntius sophore and Labeo calbasu with the aid of procedure tripsinization (Rishi and Rishi, 1978). Khuda Bukhsh et al. (1978) studied the karyomorphology of Chanda nama and Trichogaster lalius. Rishi (1978) studied the giemsa banding in Channa punctatus.

Analysis of karyotypes of six marine species were conducted by Choudhury et al. (1979) and chromosome of marine percoid fishes by Patro and Prasad (1978). Khuda Bukhsh (1979) studied the chromosome of Aplocheilus panchax, Lates calcarifer and Gadusia chapra. Banding pattern (G-bands) of somatic chromosome of Colisa fasciatus were studied by Rishi (1979). Mitotic metaphase spread from kidney tissue of Labeo rohita and L.calbasu and F1 hybrid of this two species were studied by Krishnaja and Rege (1979). Modal diploid number to be 2n=50 in all fish with NF=78 in L.rohita and NF=82 in L.calbasu and NF=80 in F1 and F2 hybrid. No sex chromosome were reported in this study.

Krishnaja and Rege (1980) have studied meiotic and mitotic chromosome preparation from various tissues such as scales, fins, gill, intestine, kidney, gonad of teleost with simple and quick method. Khuda Bukhsh (1979 and 1982), Khuda Bukhsh and Nayak (1982) reported karyomorphology of hill stream fishes. Rishi and Singh (1982) reported chromosome complements in five estuarine fishes. Chaudhury et al. (1982) reported the chromosome morphology in five species of Tetradontiforms fishes from Indian Ocean. The diploid number of these fishes ranging from 40-48 were exposed in this study.

Das (1983) reviewed the status of Cytogenetic studies in marine fishes. Manna (1983) reported that the Indian cytologist determined the number, morphology and behaviour of some 125 species of fishes belonging to 40 families of teleosts of both inland and marine waters. The progress in fish cytogenetics was extensively reviewed by Manna (1984).

The usefulness of a modified air drying method to increase mitotic metaphase spread and permanent chromosome preparation for karyotype studies in carps was mentioned by Reddy and John (1986). Barat and Khuda Bukhsh (1986) reported the karyomorphometrical studies in fishes (Lepidocephalichthys guntea and Mystus corsula).

Chromosome of freshwater teleosts were also reported by Khuda Bukhsh and Barat (1987). Nayak and Khuda Bukhsh (1988)

reported the diploid number and chromosome configuration of Platycephalus tuberculatus. Their study showed that diploid number of this fish was 48 and chromosome configuration was 2 metacentric, 2 submetacentric, 3 subtelocentric and 17 telocentric pairs. Rishi (1989) reviewed the present status of cytogenetics in India. Beginning of chromosome study, current technique, polyploidization, Robertsonian rearrangement , Non-Robertsonian rearrangement, chromosomal polymorphism, sex chromosome and important areas where cytogenetic would be helpful (i.e. monitoring genotoxic agents, induced gynogenesis hybridogenesis and induced polyploidy and hybridization) were elaborately discussed in this paper. The Importance of cytotaxonomy, evolution and monitoring genotoxic pollutant were described by Manna (1989). Recent trends in Indian fish genetics research, and induced gynogenesis, polyploidy, genetic toxicology were discussed by John and Reddy (1989).

The diploid number of Psilorhynchs succato (2n=50), Labeo dero (2n=50) and Ompok pabo (2n=154) were studied by Khuda Bukhsh and Chanda (1989). Chakraborty and Kagwade (1989) reported the diploid number of 48 acrocentric chromosome in Otolithis cuvieri and Nibea diacanthus.

Nayak and Khuda Bukhsh (1991) studied the chromosome complements of somatic tissue in Mugil corsula with C-band localization. The diploid number of this species as 48 was also reported in this

study. Khuda Bukhsh and Tiwary (1991) have determined the metaphase chromosome of Anabas testudineus by silver nitrate staining method and present status of knowledge on fish NOR has been discussed. Rishi and Manjusha (1991) opined that C-banding and NOR localization technique were better than G-banding technique to study the accurate determination of karyotype. Karyological studies of twenty five species of decapod crustacean were conducted by Ahmed and Nayak (1991). Usha (1991) opined that Crasostrea and Ostrea have same number of chromosome. Karyotype of C.gryphoides from west coast of India were reported in this study.

Sharma et al. (1992) studied the karyotype of two species viz Exostoma stoliczkae (family Sisorida) and Sizothorax richardsonii (family Cyprinidae). The diploid number and chromosome configuration of these two species are 2n=42 in E.stoliczkae, chromosome configuration is 34=metacentric, 4=submetacentric and 4=telocentric and in 2n=98 in S.richardsonii, chromosome configuration is 66=metacentric, 16=submetacentric, 6=subtelocentric, 10=telocentric. Sudheesh et al. (1992) has given the detailed description about karyomorphology of Lates calcarifer. The morphological, cytological and cytotaxonomical observation were carried out and chromosome number (n=18) of Nitella hyalinavar hyalina was recorded for the first time from India by Pundhir et al. (1993). Subbarao et al. (1994) reported the cytogenetic evidence for three sibling species of Anopheles fluviatilis.

2. GENOTOXICITY STUDIES

The genetic toxicology was probably started with the study on genome mutation induced by radiation which was demonstrated by Muller (1927), followed, almost 20 years later, by the mutagenicity study with chemicals (Auerbach, 1946). The genetic changes in animals induced by radiation and chemicals were conducted after twenty years by Cattanach (1966) and Russel (1977). The results of these studies indicated the awareness that some of the hereditary diseases observed in human populations might be environmental in origin. Kayser et al.(1962) observed the nerve cell injuries caused by organic phosphate pesticide in fishes and crab. The epizootic of liver neoplasia in fishes from area with high level of environmental pollutants were reported by Dawe, et al.(1964). The chromosome aberrations in marine fish was studied by Regan et al. (1968). Schroder (1969) studied the X-ray induced mutation in Poecilid fish, Lebistes reticulatus. The genetic effects of x-irradiation in hybrid and inbred male and female of this fish were observed in this study. Subrahmanyam (1970) investigated the use retenone, fish toxicant as a mitostatic agent useful for chromosome work. Brookes and Lawly (1971) reported the somatic and germ cells mutation. The detection of chemical mutagen with aid of microorganisms was reported by Ames (1971) and induced mutation in yeast (Mortimer and Manney , 1971 ; Dean et al.,1972; Parry, 1973). The mutagenic effect of dichlorvos on dose dependent manner in different bacteria were reported by Vooged et al.(1972) and Mohn (1973) in mice and chinese hamster by Dean and Thrope (1972, a,b). Tystsugima (1972) reported the frequency of chromosome changes in eggs of Scorpaoma sp. after chronic exposure

to radiation. Alkylation reaction of dichlorvos in Escherichia coli (Bridges et al., 1973) in fishes (Lawely et al., 1974) and its effects on disintegration of DNA in E.coli and chinese hamster (Green et al.,1974) were reported. Kligerman et al. (1975) studied the chromosome aberration in Umbra limi. Intestine, stomach, kidney and gill tissues of this species were found suitable for clastogenic studies and they reported 0.3% of spontaneous chromosome aberration and 30% of induced aberration by 325 R of X-rays. Savage (1975) described the chromosomal aberrations as a criterion for the evaluation of potential environmental hazard. The effect of industrial effluents on histopathological changes in fishes were reported by Mukherjee and Bhattachariya (1975). Parry and Evans (1975) reported the examination of response of chromosome in cultured cells to a wide variety of mutagen.

Parry et al. (1976) studied the effect of pollution on genome of mussel, Mytilus edulis. The chronic irradiation influenced on induction of chromosome aberrations in cultured cells of fish Ameca splendens were reported by Woodhead (1976). Mong and Berra (1976) reported the effects of X-radiation on the chromosome of U.limi. Chromatid gaps and chromatid exchange between several chromosomes were studied after fishes were subjected to 350R, 660R and 990R of X-radiation. The frequency of aberration per metaphase increased with radiation dose were also described in this study. Pechkurenkov (1976) opined that continuous irradiation in fish embryos causes

severe chromosome aberration. The chromosome mutagenesis of mackerel, Scomber scombrus were reported by Longwell (1976), Stich and Acton (1976) reported the tumours in fish caused due to sublethal effects of pollutants and feasibility of using this system for easily detection of mutagen in marine environment.

Microbial procedures were used to detect the mutagen and toxicants distributed in the Lake Ontario (Dutka and Switzer, 1978). The cytogenetic changes in fish after exposure to Rhine water were studied by Prein et al. (1978). Ravindran and Ravindran (1978) studied cytological irregularities induced by water pollution with factory effluents. Kligerman (1979) described the use of aquatic organisms to detect the mutagen which cause the clastogenic changes. The effects of radiation in aquatic organisms especially fishes were described by Schroder (1979). Grant (1979) elaborately reported the genotoxic effects of 2, 4, 5-T. Ahokas (1979) reported the effects of P-450 in fish liver microsomes and carcinogen activation. Barker and Rackham (1979) opined that carcinogenic mutagen induced the sister chromatid exchange in cultured fish cells. The high incidence of neoplasia in fish residing in area high in anthropogenic pollution (Kraybill, et al., 1977; Sinderman, 1979; Smith et al., 1979; Black, 1983; Murchelano and Wolke, 1985; Hendricks et al., 1985; Malins et al., 1984, 1985; Krahn et al., 1986).

The effects of some pesticides and detergents on the fish chromosome sets, as the preliminary investigation, were studied by Sofradzija et al.(1980).Newsome (1980) reported that multigeneration fish toxicity test to asses the effects of potential aquatic pollutants using Cichlasoma nigrofasciatum. Fox and Scolt (1980) reported the

genotoxicity of nitrogen and sulphur mustard. Beardmore et al. (1980) and Berry (1980) reported the genetical approaches to monitor the pollution. The need to study distribution of genotoxic substance, their effect on gene pool, and genetic damage were discussed in this report. Brusick (1980) reported the principles, origin, assays and application of genetic toxicology. Mukherjee and Manna (1980) investigated the effects of malathion on chromosome of Tilapia mossambica. Alink et al. (1980) studied the induction of sister chromatid exchange in fish exposed to Rhine water. Chronic exposure of waterborne selenium on rainbow trout (Salmo gairdneri) were studied by Hodson et al. (1980) . Pesch and Pesch (1980) reported that Neanthes arenaceodentata can be used as a model organisms to study the effects of genetic toxicants in marine environment.

Wardhaugh (1981) has studied the dominant lethal effects of mutation in Tilapia mossambica which was induced by antileukaemic drug, myleran. Hooftman (1981) studied that induction of chromosome aberrations by ethyl methane sulfonate and benzo (a) pyrene in Notobranchius rachowi. Kocan et al. (1981) reported the effects of mutagen and carcinogen on cultured fish cells.

Manna (1982) reviewed mutagenesis and described the needs to study the mutagenesis caused by living mutagen. The induced chromosomal aberration in fish, Boleophthalmus dussumieri after in vivo exposure to mytomycin C and heavy metals mercury, selenium and chromium which will increase the frequency of aberration like chromatid and isochromatid breaks, fragments, rings, exchanges and unclassified markers were studied by Krishnaja and Rege (1982)

whereas mutagenic effect of pesticides were reported by Waters et al. (1982) and Sandhu et al. (1985). Anuploidy were reported in mussel embroys collected from polluted area (Dixon, 1982). Kocan et al. (1982) observed the chromosome damage of cultured gonadial cells of rainbow trout (Salmo gairdneri) after treatment with N-methyl N-nitrosoguanidine, benzo (a) pyrene, 9 amino acridine, 3 methyl colanthene, anthracene, 1-naphthol and mytomycine. The chromosome damage like fragments, deletion, complex rearrangements rings, translocation, etc. were observed in Liposetta putnami after exposed to soluble fraction of crude oil (Bruke, 1982).

Regan et al. (1983) observed the capacity of fibroblast from two closely related marine fish, the tautoy and the cunner, for excision repair of UV and chemically induced damage in their DNA. Mitami (1983) observed the lethal and mutagenic effects of radiation and chemical on cultured gold fish Carassius auratus. Chromosomal damage in salmonid due to irradiation were reported by Yamazaki (1983). Landolt and Kocan (1983) reviewed on fish cell cytogenetics and evaluated some of the methods like Ames test, fish cell mutation, micronuclei formation and aberration for measuring genotoxicity in fishes.

Somatic chromosome aberration of the X-irradiation to tilapia were studied by Som and Manna (1984). Zajick and Philips (1984) observed the mitotic inhibition and anaphase aberrations in rainbow trout, Salmo gairdeneri after exposed to MNNG and gamma radiations. Manna and Mukherjee (1986) reported the the genotoxic effects of organophosphate insecticide, malathion. This study emphasised increased the chromosome aberrations in insecticide treated tilapia. The effect of detergent and benzene in the rainbow

trout, S gairdneri were reported by Al-Sabti (1984). Landolt and Kocan (1984) used a fish cell cytogenetic assays (anaphase aberrations) as a system for determining the genotoxic activity of organic extracts obtained from marine sediments. Grizzle et al. (1984) reported pathological effects of fishes due to chlorinated waste water effluents whereas organophosphate pesticide, monochrotophos effects on liver tissue of T.mossambica were reported by Desai et al.(1984).

Kelly and Maddock (1985) investigated the genotoxic effects of mutagenic carcinogen in marine fish. The cytogenetic changes in rainbow trout, S.gairdneri produced by phenol, decamethrine, neguvon, malathion and crude oil were studied by Al-Sabti (1985a, b) who also reported the induced chromosome aberrations in kidney cells of cyprinid by carcinogenic and mutagenic chemicals. Kocan et al. (1985 a) studied the fish cell culture used to detect the pollution in marine environment. Rainbow trout gonads and bluegill fry tissues were used to monitor the cytotoxicity and genotoxicity of the sediment extract, benzo (a) pyrene and MMNG. The cell death, mitotic inhibition, stimulatory effects and chromosome damage were evaluated in this study. Kocan et al. (1985 b) have reported that visible anaphase aberrations could predict genetic defects in rainbow trout, S.gairdneri and emphasised the relationship between visible and heritable chromosome damage. Response of cells of marine molluscs to pollutants were studied by Moore (1985). Liguori and Landolt (1985) studied the anaphase aberration in rainbow trout S.gairdneri as an in vivo measure of genotoxicity. In this study

chromosome aberration, developmental and pathological defects of this species, after exposure to N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) were reported.

Manna (1986) reviewed the genotoxicity studies in fishes and emphasised that tilapia as a suitable model for assessing the genotoxic agents. The effect of clastogenic chemicals (aflotoxin, aroclor, benzidine, benzo (a) pyrene and methylchloranthrene) on common carp, C.carpio were used to study the chromosome aberration (Al-Sabti, 1986 b). Crespo and Sala (1986) studied alteration of chloride cells of gill epithelium of the dog fish, Scyliorhinus canicula.

Shah and Beardmore (1987) observed the effect of MMS to induce the dominant lethal mutation in tilapia, Oreochromis niloticus. The chromosome fragmentation and loss in two salmon hybrid were reported by Goodier et al. (1987). Shugart et al. (1987) observed the adduct formation in the bluegill sun fish, Lepomis macrochirus. Pancorbo et al. (1987) studied the mutagenic activity of surface water adjacent to nuclear fuel processing factory.

Chen (1988) studied the toxic mechanisms of environmental xenobiotic at sublethal level and screened toxic chemical pollutants in aquatic system. The chromosome aberrations in gill, kidney and intestine of embryonic stage of two fish species such as striped bass, (Morone saxitillis) and shephead minow, (Cyprinodon variegatus)and in the adults of Fundulus heteroclitus when exposed to

chemical mutagens, including 9-aminoacridin (9AA), ethyl methane sulphonate (EMS), cyclophosphamide (CP) and N, methyl-N-nitro-N-nitrogunidine (MNNG) were reported by Means et al.(1988). Shugart (1988) used alkaline unwinding assay to study the effects of chronic exposure of genotoxic chemical benzo (a) pyrene in the liver tissue of bluegill sunfish, Lepomis macrochirus and fatheard minnows, Pimephales promelas. Black (1988) reported that the carcinogenicity test using rainbow trout S.gairdneri embroys will detect most direct acting alkylating agents such as dimeythylnitrosamine, N-methyl-N-nitro-N-nitrosoguanidine, methyl-nitrosourea. Perry et al. (1988) observed the effect of methyl mercury on cellular part of embryo of the killifish, Fundulus heteroclitus. The acute liver lesion in fish populations has been closely correlated to accumulation of certain environmental chemical contaminant (Malins et al.,1985; Kohler, 1989; Myers et al., 1991).

Kira et al. (1989) measured the mutagenicity in mussel bodies and that of their ambient water. This study has also indicated the correlation between the mussel and water mutagenicity. Manna (1989) reviewed the various protocol for genotoxicity test using fish species. Manna and Mukherjee (1989) studied the genotoxic potentiality of the inorganic weedicide, sodium arsenite in experimentally treated tilapia, Oreochromis mossambica. Stephanie Mills (1989) and Stephanie Paine(1989) reported eye disease due to nuvan in salmons. Ostrander (1992) reported retinoblastoma

following exposure of medaka. Oryzias latipes to methylazoxymethanol acetate. Goldberg et al.(1990) reported that cellular oncogenes can be activated by point mutation, deletion, insertion or rearrangement, through chromosomal translocation or gene amplification.

There are many reports regarding the isolation of oncogene and tumour suppressor genes in fishes. Isolation of myc gene (Van Beneden, et al., 1986 and ras gene (Mangold et al., 1991, and Chang et al., 1991) from rainbow trout Oncorhynchus mykiss, and from Carassius auratus. (Nemoto et al., 1986, 1987), atlantic tomcod Microgadus tomcod (Wirgin et al., 1989) and winter flounder Pseudopleuronectes americanus (Mc Mahon et al., 1990). Molven et al. (1991) reported isolation of wnt-1 gene from Zebrafish (Brachidanio rerio) whereas tumour suppressor genes p53 from rainbow trout (Soussi et al., 1990), Rb genes from medaka Oryzias latipes (Ostrander et al., 1992), coelacanth Latimeria chalumnae, rainbow trout O.mykiss, english sole Parophyrs vetulus (Van Beneden and Ostrander,1994) were reported.

Hongell (1990) conducted survey of chromosome aberrations of grey seals, Halichoeres grypus and ringed seal, Pusa hispida. Genotoxic effects of isoproturon (herbicide) was measured by employing in vivo chromosome aberrations, micronuclei and sperm head abnormality (Behera and Bhunya, 1990). Pankaj et al. (1990) studied chromosome damage in Cyprinus carpio exposed to two different concentrations (0.18 and 0.32 ppm) of cadmium nitrate at 24 hrs and 48 hrs. Significant increase of chromosome aberration were obtained

at 48 hrs exposure in both concentration. Mitrofanov et al. (1991) observed alternative process of chromosome aberrations.

The bacterium, Stephylococcus aureus was used as genotoxic agent in five species of fresh water teleost (Manna and Biswas, 1992). Manna and Mukherjee (1992) studied genotoxic effects of the insecticide, endrin in experimentally treated cichlid fish, Oreochromis mossambica.

Mahapatra and Noble (1992) studied the protein, total free amino acid, RNA and DNA and RNA ratio in muscle tissue of L. parsia. Jones et al. (1992) reported the detection of chromosome damage for assessment of exposure of genotoxic pollutants. The in vivo genotoxicity of waste water from a wheat and rye straw paper pulp factory were studied by Wrisberg and Gaag (1992). Di Gulio et al. (1993) observed the xenobiotic biotransformation,oxidative stress and DNA integrity in liver and bile of channel catfish, Ictalurus punctatus, after exposed in laboratory to sediments obtained from Black Rock Harbour . Cytological alteration in Oncorhynchus mykis exposed to 1, 3, 10 mg per litre of 4 - chloroanilin for upto 8 days were studied by Braunbeck (1993). Johnson (1993) modified the traditional rodent salmonella/microsome mutagenicity test of Ames by salmonella/fish S9 mutagenicity which was established with channel catfish, Ictalurus punctatus, as model system for monitoring genotoxin.

Kurelec (1993) suggests that genetic damage is manifest as a suit of pathophysiological changes. This comprises impaired enzyme function, metabolism, enhanced protein turnover, inhibition of growth, decrease the scope for fecundity and faster ageing. Genotoxins can induce changes in DNA that are passed on to future generations.

Trout Oncorhynchus mykis, were exposed to Rhine water for 24 days to study cellular response (Iger et al., 1994 a). Cellular response of Cyprinus carpio after exposure to 1.6 µM of copper for different period upto 43 days were also studied by Iger et al. (1994 b). Jeberge and Jensen (1994) reported the extra-cellular and intra-cellular changes in cray fish, Astacus astacus exposed to 0.5 and 1.0 mM of nitrate. Voccia et al. (1994) studied cytotoxic and immunotoxic effects of mercuric chloride and methyl mercury in O mykis. Van beneden (1994) reported that during past few decades there have been increase in both the number and type of tumors found in fin and shell fishes. Depledge (1996) extensively reviewed the literature in ecotoxicology.

MICRONUCLEI STUDIES

Micronuclei (MN) are the small cytoplasmic chromatin containing bodies arising from the chromosomal fragments or whole chromosome to appropriate spindle pole to be included in the main nucleus of daughter cells following mitosis. After telophase the undamaged chromosome as well as centric fragments give rise to regular daughter nuclei. The lagging elements are also included in daughter cells which are much smaller than the main nucleus and are therefore called the micronuclei (MN).

Howell (1891) and Jolly (1905) discovered the micronuclei in red blood cells. In early days, these micronuclei were, therefore, called Howell-Jolly bodies. The first serious attempts was made by Evans et al, (1959) and followed by Schroeder (1966, 1970). Micronuclei were used as indicator of chromosome damage in their studies which were expanded for a wide range of genotoxicity testing.

Schmid et al.(1971);Heddle(1973);Von Ledebur and Schmid (1973); Schmid (1975, 1976) reported micronuclei could be used as a monitor to find out the cytogenetic damage. They have developed in vivo test on the identification of micronuclei, MNT was used as a fast method for testing genotoxic substance in mammals (Evans, 1976, Schroeder, 1970; Heddle et al., 1983; Sutou, 1981, 1986). Modified MNT test and clastogen induced micronuclei in peripheral blood erythrocytes were studied by Mac Gregor et al. (1980). Hoofman and de Raat (1982) reported induction of nuclear anomalies and

micronucleus in peripheral blood erythrocytes of fish, Umbra pygmaea by the genotoxic compounds (ethyl methane sulphonate) for 3 to 6 week. The micronuclei formation in this species after 3 week exposure of EMS (200 mg/litre) were 1.6%. Wolters et al. (1982) studied the nuclear measurements of diploid and triploid channel catfish. Manna et al. (1985) observed the micronuclei in peripheral erythrocytes of Saratheredon mossambicus exposed to aldrin (0.3%), cadmium chloride (0.1%), D-glucosamine hydrochloride (0.1%) and X-radiation (500r) respectively. Comparison of effects of these genotoxic agents were carried out in this study. Manna and Sadhukhan (1986) studied the micronucleated cells (MNC) of gills and kidney of tilapia, Oreochromis mossambicus treated with X-rays and chemicals (cadmium chloride and D - glucosamine). They opined that cells of gills appeared more sensitive to X-rays and cadmium chloride than those of kidney and erythrocytes. Al-Sabti (1986a) studied the comparative micronucleated erythrocyte cell induction in three cyprinid by carcinogenic and mutagenic chemicals. Jaylet et al. (1986) reported the micronuclei formation in peripheral erythrocytes of axolotl larvae Ambystoma maxicanum following in vivo exposure of mutagenetic agents, benzo (a) pyrene and ethylmethene sulphonate. The 8 days exposure of B[a]P at 0.25 ppm and 10 days exposure at 0.1ppm, and 8 days treatment of EMS at 24 ppm and 4 days treatment at 62 ppm showed numerous micronuclei. The mitomycine C and paper mill effluents induction of peripheral erythrocytes of Heteropneustes fossilis were studied by Das and Nanda (1986). Benzo (a) pyrene

induced micronuclei in the red blood cells of newtt Pleurodeles wattle were studied by Grinfield et al. (1986). Hose et al. (1987) observed the elevated rate of micronuclei in the erythrocytes of fish from contaminated site of southern California. Manjone et al. (1987) opined that the increase of micronuclei in the gill tissue of marine mussel, Mytilus galloprovincialis exposed in mitomycine C. The persistence of micronuclei for long time and significant increase of micronuclei after treatment of MMC were described in this study. Chlorothalonile induced increase of micronuclei in erythrocytes cells of eels were reported by Mingde et al. (1987). The frequency of micronuclei in gill tissue of M.galloprovincialis induced by zinc chloride with different staining techniques (acridine orange, gallocyonin, chromallum, Fuelgen and Giemsa) which were compared with each other (Manjone et al., 1988). Metcalfe (1988) opined that cells with high mitotic turn over is considered for effective screening of micronucleus. Manna (1989) reviewed the different report for measuring genotoxicity using fish and stress the relevance of cytogenetics.

Carrasco et al. (1990) studied the assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Wild white croaker (Genyonemus lineatus) collected from polluted area of California coast, was used as test animals for this study. The induction of micronuclei by mitomycine C and colchicine in the mussel M.galloprovincialis were studied by Manjone et al. (1990).

Nikinmaa (1992) studied the fishes exposed to heavy metals and other environmental pollutants cause different nuclear anomalies including micronuclei. Annual cycle of blood chemistry parameters in striped mullet, Mugil cephalus and pin fish Lagodon rhombodies from the Gulf of Mexico were studied by Flomar et al. (1992). The genotoxic potential of the bacterium, Hay Bacillus. (Bacillus subtilis) has been tested at 24 hrs intraperetoneal injection of culture at the rate of 1 ml per 100 gm body weight of tilapia, Oreochromis mossambica to study the chromosome aberration and micronuclei (Biswas and Manna, 1992). In vitro micronucleus test in mammalian cells and aquatic species like Mytilus were studied by Gabriele et al. (1992). Rahman and Khuda Bukhsh (1992) studied the genotoxic potential of industrial effluents (from Paper pulp and viscose Rayon factories released in the Hooghly river) and mercuric chloride, stannous chloride and phenol were assessed by micronuclei testing (MNT) of peripheral erythrocytes in the fishes Oreochromis mossambica. Channa Punctatus was used only for mercuric chloride treatment in this study. Nusse et al. (1992) conducted experiments to study the induction of micronuclei by 2 chlorobenzlidene malonitrite. The temporal pattern of base line frequency of micronuclei in M. galloprovincialis were studied by Brunetti et al. (1992). Williams and Metcalfe (1992) developed two assay protocols to study the genotoxicity of ethylmethane sulphonate (EMS), mitomycine C (MMC), diethylnitrosamine (DEN) were used to study in vivo hepatic micronucleus assays with Oncorhynchus mykis. The measurement of

the production of chromosome structural and numerical aberrations using micronucleus assays were conducted by Jones and Parry (1992).

Miller and Nusse (1993) observed the frequency of induced micronuclei by 2-chlorobenzylidine malonitrite using fluorescence in situ hibridization (FISH) technique.

Behari et al.(1994) conducted an in vivo study on effect of physical and chemical genotoxic agents. The cat fish, Clarias gariepinus was irradiated at dose of 0-9 Gy or intraperitoneal injection of mitomycine C at concentration of 0-2 mg/kg for 0-60 days treatment to study the induction of micronucleated erythrocytes. Al-Sabti et al. (1994) investigated the effects of chromium (Cr VI and Cr VIII) in Prussian carp, Carassius auratus gibelio for detecting the cytogenetic damage and micronucleus induction in erythrocytes cells. Cytogenetic damage and micronucleus were also studied in C. auratus collected from polluted area.

3. TOXICITY STUDIES

In recent years the pollution in aquatic environment has been increased many a fold. The hazards of these pollution (pesticides, industrial effluents, sewage, etc) have been found to be highly toxic not only to fishes but also to other aquatic organisms which constitute the food of the fishes (Anderson, 1960; Loosanoff, 1960). Durhama and Williams (1972) also reported pesticides are widely used in agriculture. These pesticides may enter aquatic system indirectly by drift from spraying of agriculture land, air dust particle, accumulated in the atmosphere, run off from agriculture land, effluents from industries and factories, etc. The pesticides are known to produce cumulative deleterious effects on fishes and other aquatic organisms inhabiting the environment. Duke and Dumas (1974) reported that in aquatic environment there was possibility of precipitation of pesticides at the area of estuaries. Among the pesticides, organophosphorous compounds are widely used in agriculture sector. Butler (1971) opined that the effect of organophosphate pesticides appeared to be higher in marine crustacean than that of organochlorine. Trip (1974) reported the effects of organophosphate pesticides in Crassostrea verginica.

The inhibition of acetyl cholinesterase (AChE) enzymes which are in part essential for normal neural transmission, due to organophosphate pesticides has been well established in vivo and in vitro in fishes (Weiss, 1958, 1959, 1961, 1964, 1965); in fishes from polluted sites (Williams and Sova, 1966); in mammals and insects (Wild, 1975). According to Eistler(1969) many of the insecticides are hazardous to the freshwater, estuarine and marine organisms at extremely low concentration. Sprague (1969) reported

the methods to measure the level of pollutants in aquatic animals. Many workers reported that pesticides can induce behavioural change in fishes. The convulsive activity just before death of salmon and trout exposed to higher concentrations were reported with DDT by Kerswill and Edwards (1967); with fungicide 'nabam' and with insecticide 'endrin ' by Saunders (1969).

Although organophosphate pesticides breakdown more rapidly than chlorinated hydrocarbon, there is evidence that, they may retain their toxicity as the parent compound or as a hydrolysed breakdown product for a longer period (Cowart et al., 1971 and Faust and Gomma, 1972). An organophosphate pesticide fenitrothion at a concentration of 1 ppm induced aggressive and territorial behaviour in coho salmon Oncorhynchus kisutch (Bull, 1971), and in Salmo salar increased suceptibility to predation by brook trout Salvelinus fontinalis (Hatfield and Anderson, 1972), and impaired the learning process (Hatfield and Johansen, 1972) and inhibited the holding territorial behaviour (Symons, 1973). Welsh and Hanselka (1972) described the behavioural changes in siamese fighting fish Betta splendens induced by methyl parathion. Rand et al., (1975) reported the effects of parathion in the locomoter orientation of gold fish Carassius auratus. The changes in behaviour of Cyprinus carpio communis after exposure to malathion were reported by Toor et al. (1973) and Toor and Kaur (1974).

Coppage and Matthews (1974) reported the mechanism of acetylcholinesterase inhibition in pink shrimp by malathion whereas its effects on brown and white shrimp in field were reported by Conte and

Parker (1975) and this pesticide effects on inhibition of oxygen uptake in fishes were reported by Ranke - Rybika (1975). Micro-organisms, especially bacteria were also used as model organisms to study mutagenic pollutants (Ames et al.,1975). Unnithan et al.(1975) studied the organic pollution at six polluted sites (Padiyathukulam canal, canal at Ernakulam market, Kalavathi canal at Fort Cochin, Rameswaram canal at Mattancherry, Pulimuttu canal at Palluruthy and Thevara canal in Cochin back water.

Tagatz (1976) reported that exposure to mirex, an organochlorine, resulted in greater predation on grass shrimp by pin fish Lagoden rhomboides. The presistence of aquatic pollution could lead to long term effects on benthic communities (EPA 1976). Desi et al. (1976) conducted toxicity studies that the effects of malathion on mammals, aquatic organism and tissue culture cells. The water pollution and fish mortality in Cochin backwaters were studied by Silas and Pillai (1976). The Methyl mercuric chloride on three generation of brook trout Salvelinus fontinalis were studied by Mc Kim et al. (1976). Methyl mercuric chloride (MMC) of 2.93, 0.93. 0.29, 0.09, 0.03 and 0.01 mg Hg/litre concentrations were used in 144 week experiment. No toxic symptoms were observed in third generation at the lowest concentration. Sobels (1976) and Deserres (1976) indicated the need for evaluation of environmental mutagens.

Srivastava et al. (1977) studied the sublethal effects of methyl parathion on Colisa fasciata. An oil pollution induced in the Benzo (a) Pyrene Monoxygenase in marine fishes were studied by Kurelec et al.(1977) Sublethal doses of parathion were used on Palaemonetes pugio to study the impairments of antipredatory behaviour of this species (Farr, 1977). Ahamed et al. (1978) studied the effects of malathion on free aminoacid, total protein, glycogen and enzymes of Lamelliden marginalitis. The effects of fenitrothion on reproduction of Cyprinus carpio communis were reported by Kapur et al. (1978).

Sastry and Malik (1979) reported the effects of phosphamidon (Dimecron) on the digestive system of freshwater fish, Channa punctatus. Trace metal changed the RNA/DNA ratio of yellow perch, Perca flavescens as reported by Kearas and Atchinson (1979). The biochemical changes due to malathion in catfish, Clarias batrachus were reported by Mukhopadhyay and Dehadrai (1980) and its effects on liver of Brachiydanio rerio were reported by Kumar and Ansari (1986).

Rao (1974), Anees (1975); Sailatha et al. (1981); Pal (1983). Verma et al. (1984) and Ravikumar and Gupta (1988) have studied the level of toxic effects of organophosphate chemical on fishes.

The acute toxicity of organophosphate were reported by many authors (Matton and Laham, 1969; Symons , 1973 ; Shakoori et al., 1976; Konar, 1977; Lingaraj and Venugopal, 1978; Shaffi , 1980; Qureshi et al. 1983; Rashatwar and Ilyas, 1984; Bash et al. 1984; Pal and Konar, 1985 and Bashamohideen et al., 1987).

Nagaratnamma and Ramamurthy (1981) studied the comparative effect of methyl parathion to leech, Poecilobdella granulosa; crab, Oziolelphusa senex; mussel Lamelliden marginalitis , snail, Pila globsa and common carp, Cyprinus carpio. Zahn et al. (1981) studied the consequence of marine pollution by hydrocarbons with help of sponges Tethya iyneurium as a model organism. Murthy et al. (1981) observed the relative toxicity of the technical grade material, isomers and formulation of endosulfan to the fish, Channa punctatus. The effect of malathion on the teleost, tilapia were studied by Kabeer Ahamed et al. (1981), and Ramalingam and Ramalingam (1982) studied malathion, DDT and mercury effluents effect on this species.

Nammalwar (1984) studied the biochemical changes resulting from bioassay of pesticides, DDT and BHC to the estuarine mullet Liza macrolepis. Rao et al. (1984) observed the effects of phosphamidon, malathion and lindane on Oreochromis mossambica, which indicate that the fish is more susceptible to lindane than other two compounds and elevation in blood glucose level of fish under all the three insecticidal exposure, and effects of malathion and

phosphamidon on Channa striatus were studied by Chaudhuri et al. (1984). Sharma et al. (1983) studied the effects of malathion on mortality of fishes. Sasibushan Rao and Venugopal (1984) have studied the effect of lethal and sublethal lindane on oxygen consumption in juveniles of Mugil cephalus. Sublethal toxicity of phosphamidon on Penaeus indicus and Metapenaeus monoceros were studied by Dasarathramaiah (1984). Mani and Konar (1984) studied the LC 50 and LC 95 of malathion for fish, Tilapia mossambica ranging from 9.2 to 16.7 ppm, for plankton, Diaptomus forbesi from 0.01 to 0.54 ppm and for worm Brachiura sowerbyi from 0.05 to 0.66 ppm. Polychlorinated biphenyl effect on cultured cells of rainbow trout and human were observed by Denizeau and Marion (1984).

Reddy et al. (1985) have conducted experiment to observe the toxicity of phosphamidon in Penaeus indicus. Carbohydrate, glycogen, lactate dehydrogenase and succinate dehydrogenase decreased, in treated organisms. Dichlorvos effects in freshwater prawn, Macrobrachium lamarrei were studied by Shukla and Shukla (1985). The effects of oil on the biochemical structure of fishes were studied by Stueber and Zahn (1985).

Various investigators have reported the effect of pesticides on reproductive parameters in fishes such as ovarian development in Salvelinus fontinalis (Macek, 1969); atresia of oocytes and LH-induced in vitro ovulation in Oryzias latipes (Hirose, 1975); activity of steroidogenic enzymes in Clarias batrachus (Singh and Singh, 1985); vitellogenesis in S.gairdneri (Chen et al., 1986); ovarian steroidogenesis in C.batrachus (Singh and Singh, 1987); Channa punctatus (Inbaraj and Haider, 1988). Wester et al.(1985), Pal and Konar (1985b) also reported that the effects of pesticide may be acute, resulting in mass mortality or chronic involving changes in survival, growth and reproduction in fishes. Dethlefsen and Tiews (1985), Wester et al.(1988), Den Hartog et al.(1992) reviewed the reproductive failure and death of fishes due to exposure to pollutants.

Balachand and Nambisan (1986) studied the effects of effluents discharged from the newsprint factory into Muvattupuzha river which is connected with Cochin backwater. The harmful effects of pesticide on mussel, lobster, crab, zooplankton, phytoplankton and herrings were reported by Egidius and Moster (1987), Cusack and Johnson (1990) and Mc Henery et al.(1991). Somasundaram et al(1987) and Galli et al.(1994) have reported that breakdown products of organophosphorus are more persistent than its parental compounds and their aquatic toxicity might be of significance. Quasim et al.(1988) studied the domestic sewage, industrial waste and oil pollution in Arabian Sea and Bay of Bengal. Capel et al.(1988) reported the disappearance of fish population as well as other aquatic organisms in Rhine water due to

pollution. The harmful effects of pesticide, dichlorvos to slamon were reported by Ross (1989) and to rainbow trout by Hoy et al.(1991).

Pillai (1990) reported concentration of various pollutants occuring in the Cochin estuary. The effect of cadmium, nickel, chromium, lead and mercury on primary productivity off Cochin were reported by Kaladharan et al.(1990). Ramamurthy (1991) described the effect of oil pollution on ecology and fishery of Arabian Sea and its origin. Marty et al.(1991) have used embryo and larvae of Medaka (Oryzias latipes) as a model organism to study the toxicity of pesticide disposal site. Malathion showed lesser toxic effects to Salmo gairdneri in comparison to γ-BHC (Lindstrom-seppa and Oikari, 1990). Singh and Singh (1992) studied the effect of sublethal concentration of organophosphorus (malathion) and organochlorine (γ-BHC) pesticides on plasma level of sex steroids of Heteropneustes fossilis. Medda et al. (1992) reported the effects of Dichlorvos (nuvan) on biochemical parameters (total protein, alkaline phosphate activity and glycogen) of Labeo rohita and Cirrhinus mrigala. Paalman and Vander Weijden (1992), reported heavy metals (copper and cadmium) may leads to impairment of reproduction and mortality of fishes. The effect of pesticide on the immune system of Oreochromis mossambicus was reported by Rajavarthini and Michael (1996).

MATERIALS AND METHODS

The present study was carried out at the Central Marine Fisheries Research Institute under the Post Graduate Programme in Mariculture from January 1992 to January 1995.

1. SCREENING OF TEST ANIMALS

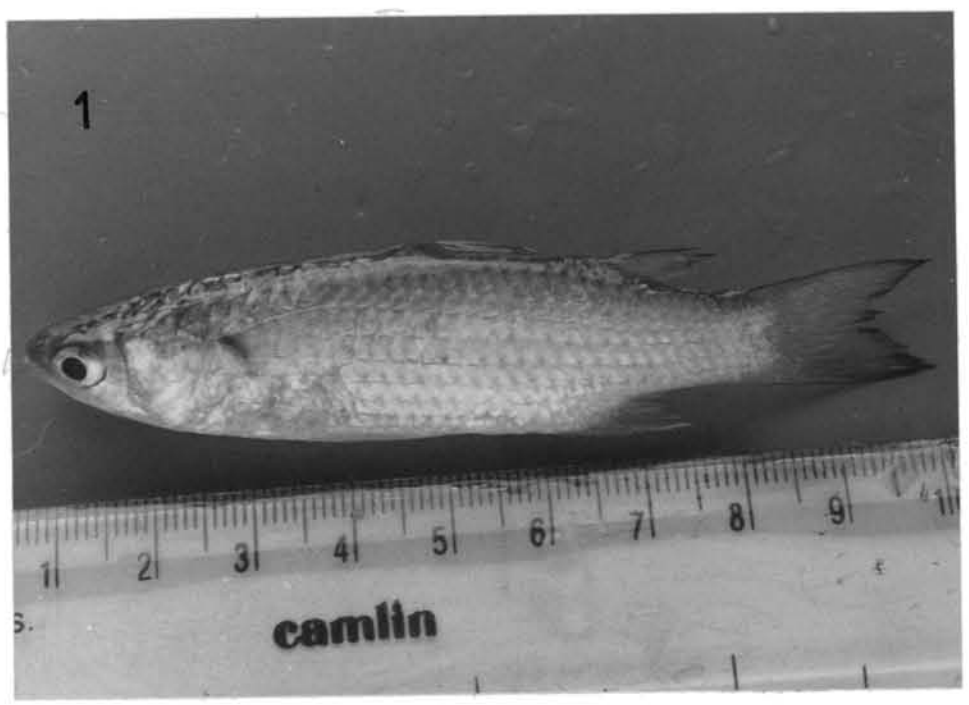
Screening of suitable candidate species is essential because candidate species should have some characteristics for this type of study Lates calcarifer, Scatophagus argus, Liza parsia and Mugil cephalus were used to screen for suitable species. Of these, L.parsia and M.cephalus showed suitable characters as model fish to study the genotoxicity. (Plate 1 and 2).

2. NATURE OF TEST ANIMALS

Mullets (Liza parsia and Mugil cephalus, family Mugilidae) were selected as candidate species for the present study. Mullets are mainly distributed in tropical and subtropical waters around the world, and few of them occur in the temperate zones. These two species are distributed in east and west coast of India. Adults and young ones are available in the sea and juveniles are found in brackishwater area. The frys feed predominantly on diatoms and epiphytes. Adults species are benthic feeders consuming algae and vegetable debris. Mullets are

Plate 1. Liza parsia (test species)

Plate 2. Mugil cephalus (test species)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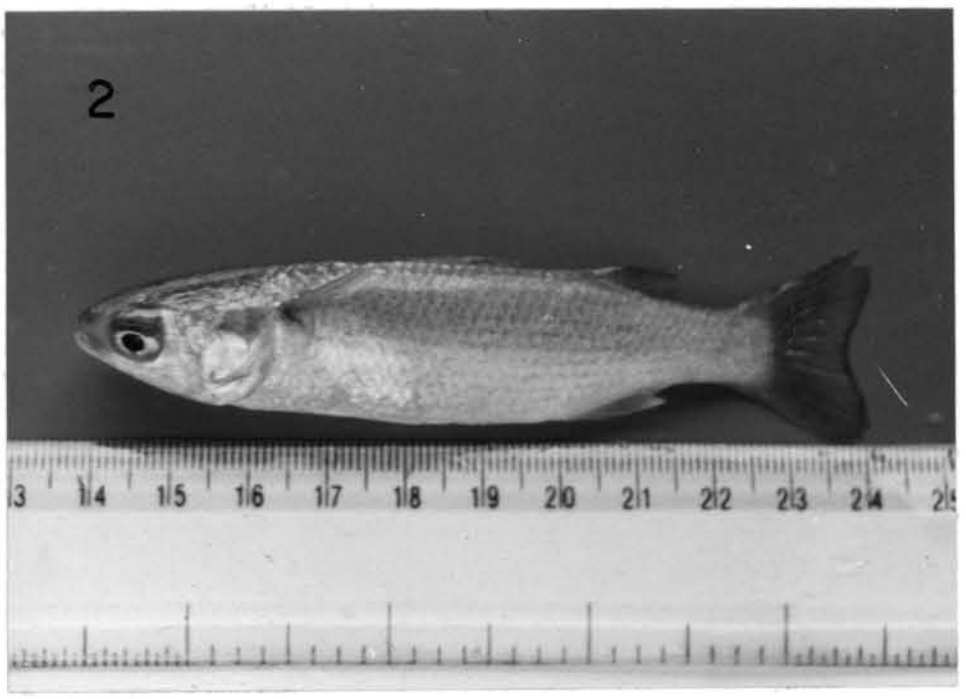
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widely cultured in Taiwan, Italy, Israel, Mediterranean lagoons, northern China, Gangetic estuaries in India, Tambak of Hava (Indonesia), coastal ponds of Hawai, etc.

Liza parsia: This fish, known as the 'Golden eye spot', is a euryhaline species which is commonly called 'Kanambu' by local fisherman of Kerala. Jhingran (1991) reported that L.parsia forms a good fishery in the estuaries of India. The peak season of occurrence of this species in Cochin backwaters is October to May with peak spawning period between December to April (Kurup and Samuel, 1983).

Mugil cephalus: The grey mullet is a marine fish, with catadromous migration, which is commonly called 'Thirutha' by local fisherman of Kerala. This species is generally caught in Chinese dipnet and is a thriving fishery in other parts of India. Peak season of occurrence of M.cephalus in Cochin backwaters is November to February. During summer season, the young ones migrate towards the less saline areas of Cochin backwaters.

L.parsia and M.cephalus are considered to be suitable species for genotoxicity studies, because they are available in polluted and nonpolluted areas. They have small chromosome number (2n=48) and can be acclimatised to laboratory condition.

3. COLLECTION AND TRANSPORTATION OF TEST ANIMALS

Live fishes (L.parsia and M.cephalus) of size 100 mm to 150 mm were collected from fish ponds at Narakkal by castnet for chromosome preparation of control and pesticide treatment in laboratory(Plate 3).Soon after the collection,the specimens were transported in suitable containers to the laboratory. About 4 to 6 fishes were transported in 10-15 litre capacity transportation bag with two third of brackishwater and ice blocks to reduce the water temperature. Transportations were made during early morning and late evening. Acclimatisation of test animals was done in 20 to 25 days time. One to two ton capacity plastic pools with biological filter were used as containers to acclimatise the fishes which were kept in brackishwater of 2-5 ppt salinity, having 3-5 ml/litre of dissolved oxygen, at room temperature. They were fed on live filamentous algae in the morning and evening.

4. CHROMOSOME PREPARATION

Two methods are generally adopted in the chromosome preparations namely the in vivo and the in vitro methods. The in vitro chromosome preparation is generally done in higher vertebrate whereas the in vivo method is more suitable in fishes because they can be sacrificed. Standardisation of methodology of chromosome preparation is to be made because the methodology may vary from

Plate 3. Collection site of fishes.


3

species to species, seasons to seasons or even laboratory to laboratory. Clear pictures of chromosomes are required to study the structural changes of chromosomes.

Various methods like Mc Phail and Jones (1966); Chen and Ebeling (1968); Denton and Howell (1969); Kligerman and Bloom (1977) and Reddy and John (1986) were tried and slight modification was made to obtain best metaphase spread of L.parsia and M.cephalus.

(a) Mc Phail and Jones (1966)

Intramuscular injection of 0.1 ml of 0.01% - 0.05% (depending on the size of fish) colchicine or velban was given to the fishes and they were then kept in a well aerated container for 1-2 hr. Posterior part of gills was dissected and was placed in distilled water for 30 minutes. The dissected gills were fixed in alcohol-acetic acid (3:1) and stained in filtered aceto-orcine for 15 minutes. The stained gills were shaken tightly on clear slides until a light slurry of cells were deposited on them. The slurry was squashed immediately using a cover glass.

(b) Chen and Ebeling (1968)

Gill epithelium of specimens were dissected out after live fish were either maintained in an aerated 0.01% colchicine solution for about

two days or injected intraperitonially with 0.5% colchicine. The removed tissues were then hypotonised with 0.9% of sodium citrate solution for 15-30 minutes. The hypotonised tissue was fixed in acetic acid and chilled acid:alcohol (1:3). Aceto- Orcine was used for staining.

(c) Denton and Howell (1969)

Freshly collected fishes were allowed to swim in well aerated colchicine solution (0.01%) in laboratory aquaria for four to five hours. The scales were scrapped and placed in hypotonic solution like potassium chloride for one hour. The swelled scales were then placed in a dish containing freshly prepared methanol-glacial acetic acid (3:1) fixative for 5 minutes and the fixative was removed and fresh fixative was added and kept for 5 to 10 minutes. Several scales were removed from the fixative and dabbed in to a clear slide to remove adherent cells. Large tissues and scales were quickly removed from the slide surface and the resulting slurry of cells was air dried for at least 10 minutes. This air dried slurry was stained with freshly prepared and filtered 2% aceto-orcine for five minutes. The slides were immediately examined for chromosome spread.

(d) Kligerman and Bloom (1977)

The gill arches, scales, kidneys and intestinal tract were removed from fishes after fishes were allowed to swim in 0.005-0.1%

colchicine solution for 6 to 7 hours, or if the fish is large enough it was inject with 25 µg/g of colcemid or colchicine. The tissues were fixed in ethanol acetic acid (3:1) with three changed at interval of 30 minutes after hypotonising the tissue with 4% Potassium chloride for 20 to 30 minutes. Fixed tissue was stored at 4°C and then removed from the fixative and excess fixative was removed using a filter paper. The tissue was immediately placed in a cavity slide and 2 to 3 drops of 50% acetic acid poured over it. The tissue were then minced for about one minute to form a cell suspension with two to four drops of 50% acetic acid. The tissue suspension was immediately drawn back and spread on a clear slide heated between 40°C to 50°C on a slide warmer. Air dried slides were then stained in 4% Giemsa stain which was prepared in 0.01 M phosphate buffer at pH 7. The slides were rinsed in distilled water after 15 to 20 minutes and placed in xylene for 10 minutes.

(e) Reddy and John (1986)

0.05% Colchicine (0.5 ml/100 gm of body weight of fish) was intramuscularly injected and after 3 to 4 hours the kidney tissue was removed which was then hypotonised with 1% of sodium citrate for 30 minutes. Hypotonised tissue was then homogenised and centrifuged and fixed in Methanol-Acetic acid (3:1). Alcohol chilled slides was used for dropping and slide preparation. The cell suspension deposited slides were then stained with Giemsa.

The following modifications were made from the above mentioned methods:-

a) Fish rearing: About 20-25 number of fishes collected were acclimatised in laboratory conditions for about 20-25 days. Salinity range of 4 to 5 ppt, dissolved oxygen of 3-5 ml/litre and temperature of 25-28°C were maintained in water for acclimatising the collected specimens, which were kept in plastic pools. Faecal matters and other debris were removed in the morning and evening. About 3-5 number fishes were maintained in well aerated water in 40 litre perspex tanks for chromosome preparation.

b) Colchicine administration: Colchicine solution in water at different concentrations 1 to 0.001% were tried @ 1 ml/100 gm body weight of fish. Intramuscular injections were given. Sufficient number of metaphases were retained after 2½ to 3 hours of treatment with 1, 0.1, 0.5% colchicine. Fishes, which were actively swimming after colchicine injection could produce large number of plates.

c) Tissues: Gill, liver and kidney tissues were taken for chromosome preparation. Gill tissue was found to be the best because they gave the best spread of metaphase followed by kidney and liver. Hence gills were chosen for this study.

d) Hypotonic treatment: Sodium citrate (0.4%, 0.8%, 1%, 2% and 5% solution in water) and potassium chloride (0.4%, 0.8%, 1%, 2% and

5% solution in water) were tried for hypotonic treatment for 15, 30, 45 and 60 minutes duration. Hypotonisation was conducted in cold as well as room temperature. The best results were obtained in cold condition.

e) Fixation: Methanol-acetic acid (3:1) was used as fixative with three change at intervals of 15 minutes, 30 minutes and 1 hour. Fixation was carried out at room temperature and cold condition. Over night cold condition fixation was also conducted.

f) Cell suspension: The chopped tissue was placed in 20-50% glacial acetic acid after the methanol was evaporated and the cell suspension was prepared.

g. Staining: 2-6% of giemsa solution in 0.1 M phosphate buffer (pH 6.8) was used for staining the slides. The staining was done for 15, 20, 25 and 30 minutes.

5. METHOD DEVELOPED FOR IN VIVO CHROMOSOME PREPARATION

Based on above trials of various steps, the following technique was developed for chromosome preparation from candidate species (L.parsia and M.cephalus)

1. The fish was given an intramuscular injection of 0.01% of colchicine solution @ 1 ml/100 gm of body weight.
2. The injected specimen was kept in a well aerated container for two and half hours to three hours.
3. The specimen was sacrificed and the tissues were removed.
4. The tissues were hypotonised for 30 minutes in sodium citrate (1%).
5. Methanol-acetic acid (3:1) was used as fixative with 2 to 3 changes (after 15 minutes, after 30 minutes and after 1 hour). Total duration of fixation was 2 hours.
6. Fixed tissue was placed in freshly prepared 40% acetic acid solution for 3 minute after allowing the methanol to evaporate from tissue.
7. Dropping the tissue suspension on warm slide (40-50°C) using pasteur pipette and by withdrawing the drop with the pipette. 2 to 4 ring of cell suspension were deposited in each slide.
8. Slides were stained with 4% Giemsa solution in a 0.1 M phosphate buffer (pH 6.8) for 18 to 20 minutes.

This standardised method was used for all the experiments during the study. Good number of metaphase plates were obtained from gill tissues.

6. PREPARATION OF KARYOTYPES

Well spread metaphase plates containing chromosomes of similar

lengths of each species were chosen for Karyotype. They were photographed and prints of same enlargement were taken along with the scale. The visually paired individual chromosomes from a plate were cut out and pasted on the white board paper.

Methodology of Levan et al. (1964) was used to classify the chromosomes. Chromosome length and its standard deviation, relative length (%) were measured from metaphase plate separately. Arm ratio of the chromosome of each species were not measured because all chromosome are acrocentric. Histograms of chromosome in good metaphase plates of each species were also made (Fig. 3 and 4).

7. METHODOLOGY FOR MICRONUCLEI

Schemid (1975) reported the micronuclei test as an in vivo cytogenetic screening procedure for the detection of freshly induced structural chromosome aberrations and for revealing chromosome loss due to partial impairments of the spindle apparatus. Micronuclei are small incomplete nuclei originated from chromatin materials which lag during anaphase. This material is included in the cytoplasm of one of the daughter cells where it can either fuse with main nucleus or form one or several main nuclei or form one or several secondary nuclei during the course of subsequent division. Micronuclei test (MNT) is one of the methods used in bone marrow cells of mammals for screening mutagenic potentiality of odd agents which has been deployed recently

in some mutagen treated fishes using peripheral blood smear (Manna et al., 1986).

Several methods were tried to find out the suitable method for micronuclei in candidate species. The following method were tried for slide preparation and staining before adopting the final one used for routine screening.

a) Schemid (1976)

Blood was collected and smears were prepared in clean slide. The smeared slides were then kept in a clean dark slide box for a day. These slides were then stained with undiluted May-Grunwald solution which was prepared with phosphate buffer (pH 6.8) in 1:1 proportion for 3 minutes followed by Giemsa staining for 10 minutes. These stained slides were then washed with tap water.

b) Salamone et al. (1980)

The slides were fixed in absolute methanol for five minutes after blood smears were prepared in slide. These slides were then stained for five minutes in 5% Giemsa solution which was prepared in 0.01 M phosphate buffer and used for screening the micronuclei.

c) Hooftman and de Raat (1982)

Blood smears were prepared and slides were fixed in methanol and stained in Fuelgen as follows. The smeared slides were treated in 1 N.Hcl for 15 minutes at 60°C and again treated with cold 1 N.Hcl and then washed with distilled water. Then they were stained for 1 hour at room temperature in Schiff's solution and then rinsed three time with freshly prepared bisulphite solution (10% $K_2 S_2 O_5$, 5 ml IN Hcl to 100 ml double distilled water) 1 % aquous light green solution was used for 1 minute as counter stain. These slides were used for screening in nuclear lesion study after being rinsed the stained slide with distilled water and dried.

d) Walton et al. (1984)

Blood smears were fixed immediately in ethanol acetic acid (3:1) for 10 minutes. 2% aceto-Orcine which was prepared by dissolving 2 gm of Orcine in 45 ml glacial acetic acid and 55 ml of Sorenson's buffer (pH 6.8), was used the staining the slides for 3 minutes. The slides were then rinsed with double distilled water and dried for observation of micronuclei.

e) Manna and Sadhukhan (1986)

Kidney and gill of fish were used which were kept in sodium citrate solution, separately minced and centrifuged at 1000 rpm for 5 minutes. The precipitated cells were smeared on clean slides and allowed to dry over night in air. Two types of staining were followed. In one of the staining methods, the slides were stained with Fuelgen stain after drying, 1 hour after hydrolyzing in 1 N HCl at 60°C for 10 minutes. In the other staining method, the slides were stained with wright stain containing methylene blue and eosin for 12 minutes and the stained slides were washed thoroughly with distilled water, dried in air and mounted in DPX for observation.

f) Hose et al.(1987)

Collected blood was immediately smeared on clean slides and allowed to air dry. These smeared slides were fixed in absolute methanol for 20 minutes and stained with May-Grunwald-Giemsa (Stock May-Grunwald and 3% Giemsa). They were washed with distilled water and then allowed to air dry.

8. METHOD DEVELOPED FOR MICRONUCLEI

The following technique was developed for micronuclei of tested animals with slight modification of above methods.

1. The blood was collected from caudal fin region by a neat cut and smeared on 5 to 7 clean slide.
2. Slides were kept in a dark place to avoid light reaction and allowed to dry over night.
3. Smeared slides were fixed in absolute methanol for 10-12 minutes.
4. The smeared slides were stained in May-Grunwald solution followed by 2% Giemsa in 0.1 M Sorenson's buffer (pH 6.8) for 15-20 minutes.
5. Then these slides were washed with tap water.
6. Observation of micronuclei was conducted after air drying the slides.

9. TEST CHEMICALS

a). Methyl Parathion 50% EC (Metacid-50)

Methyl Parathion is an organophosphorous pesticide which is widely used in agriculture in Kerala and is manufactured by Bayer A.G. Leverkusen Company. This pesticide is easily mixed in water and is already reported that it is a fish toxicant. Lethal concentration-50 (L.C.50) value of this pesticide is greater than 10 ppm in 48 hrs. Chemical name of this pesticide is 0, 0-dimethyl 0-(P.nitrophenyl) phosphorothioate.

b). Phosphamidon 85% SL (Dimecron)

Chemical name of this organophosphorous pesticide is 2-chloro-2 diethyl carbonyl-1 methyl vinyl-dimethyl phosphate which is easily soluble in water and is extensively used in agriculture. This pesticide is manufactured by Hindustan Ciba Geigy Limited.

c) Dichlorvos 76% EC (Nuvan)

Chemical name of this pesticide is 0, 0-dimethyl 2, 2 dichloro vinyl dimethyl phosphate which is easily miscible in water and is manufactured by Hindustan Ciba Geigy Limited.

d) Monocrotophos 36% SL (Nuvacron)

The chemical name of Monocrotophos, a water soluble organophosphorous pesticide , is 0, 0, Dimethyl carbomyl 1-Methyl-Vinyl-Phosphate. This pesticide also manufactured by Hindustan Ciba Geigy Limited and widely used in agriculture in Kerala.

e) Malathion 50% EC

Malathion is an organophosphorous pesticide and is soluble in water. Malathion is extensively used in paddy fields in Kerala. The chemical name of this pesticide is 0, 0, Dimethyl-S-(1, 2-di (ethoxy carbonyl) ethyl) phosphorothidate and is manufactured by S.N. Chemical Industries, Anu Product Ltd.

10 TREATMENTS

Collection and Transportation of test animals (L.parsia and M.cephalus) were carried out as in the case of control animals.

Five organophosphorous pesticides were selected for treatments. Sublethal doses were selected on the basis of tolerance limits of each chemical used. The maximum tolerated dose for 96 hours was considered as the highest dose in each case. In each case, three doses (lowest, middle and highest) were tried. In each case of the five pesticides (methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion), the doses were 0.01, 0.1 and 0.2 ppm respectively. These pesticides were administered through the water medium.

Perspex tanks of 40 litres capacity were used for the treatments with 5 animals in each tank. Two control tanks containing 5 animals in each were also maintained. The treatments were conducted in acclimatised water. The same experimental set up was used in all pesticide treatment to study the induced chromosome aberrations micronuclei formation and behavioural changes in L.parsia and M.cephalus. The methods which were developed in the present study, were used to study the chromosome aberrations and micronuclei formation in pesticides treated animals and control fishes.

11. ANALYSIS OF DATA

Data from chromosome aberrations and micronuclei formation in different individuals were analysed. Z-test was applied to analysis of data from chromosome aberrations and micronuclei formation in different individuals from treatments and control

R E S U L T S

1. DIPLOID NUMBERS

L.parsia: The diploid number of L.parsia was confirmed after examining 344 metaphases. Since significant mode value was obtained in 48 number, diploid number (2n) of this species was confirmed to be 48. The variation in number of chromosome observed may be due to cell rupture during the slide preparation (Table 1 & 2, Fig.1).

M.cephalus: In this species 300 metaphases were examined to confirm the diploid number (2n), which was found to be 48. This diploid number (2n=48) was observed in the maximum number of cases (Table 3 & 4, Fig.2).

2. ANALYSIS OF KARYOTYPES

L.parsia: In this species the chromosome complement was found to be homogenous. All chromosome were acrocentric. The size of the chromosome ranged from 4.800 to 2.125 μ. The relative length ranged from 5.332 to 2.361%. No heteromorphic sex chromosomes were observed. (Table 5, Fig.3 and Plate 4).

M.cephalus: Karyotype analyses showed that all the chromosomes were of acrocentric type. The size of the chromosome ranged from 5.5280 to 2.505 μ. The relative length in this species ranged from 6.402 to 2.606% (Table 6, Fig.4, and Plate 5).

Plate 4. Karyotype of L.parsia (Description in photograph)

Plate 5. Karyotype of M.cephalus (Description in photograph)

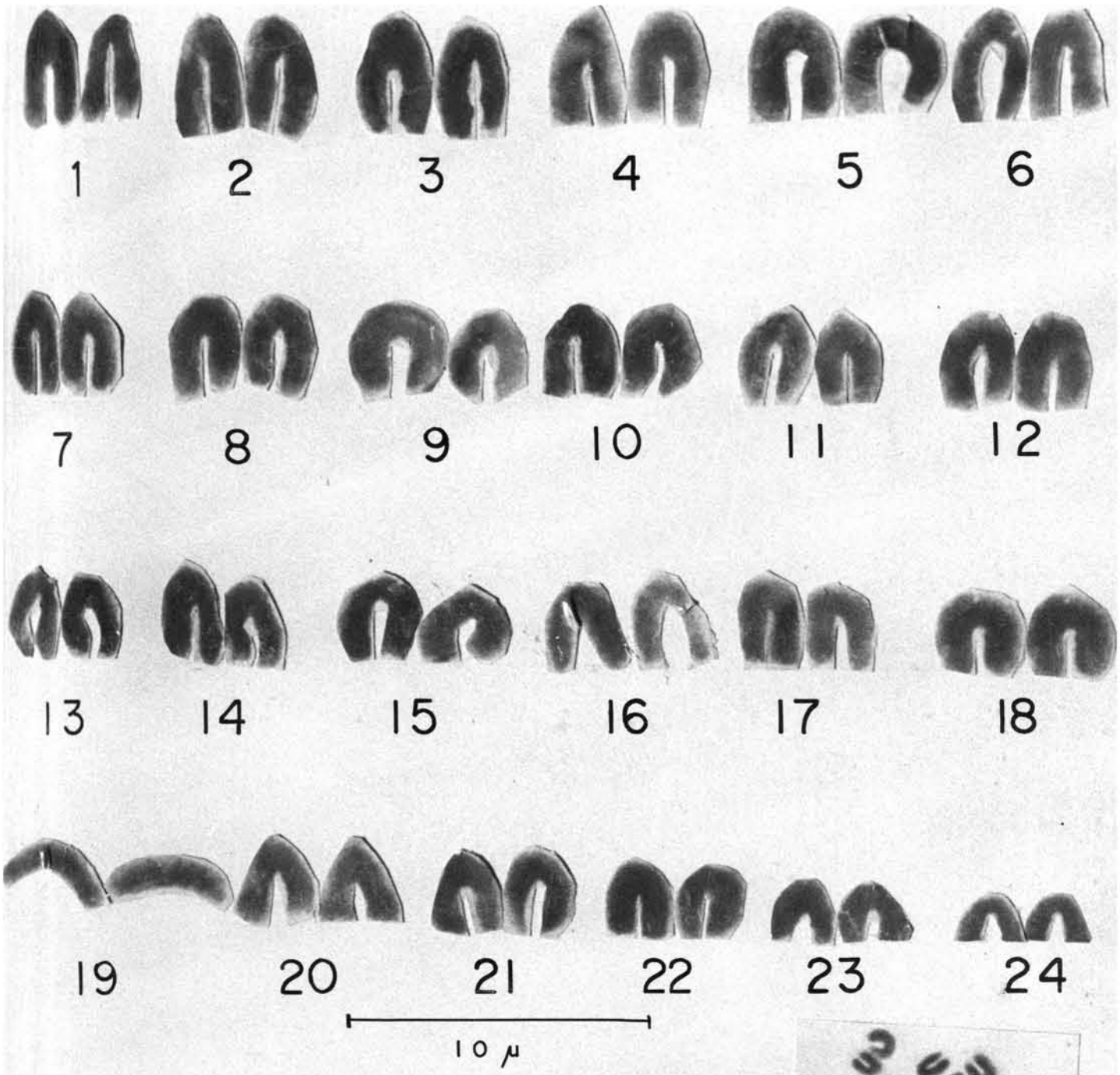
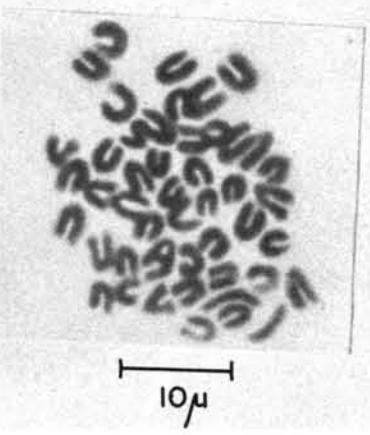


Plate – 4

Karyotype of L. parsia



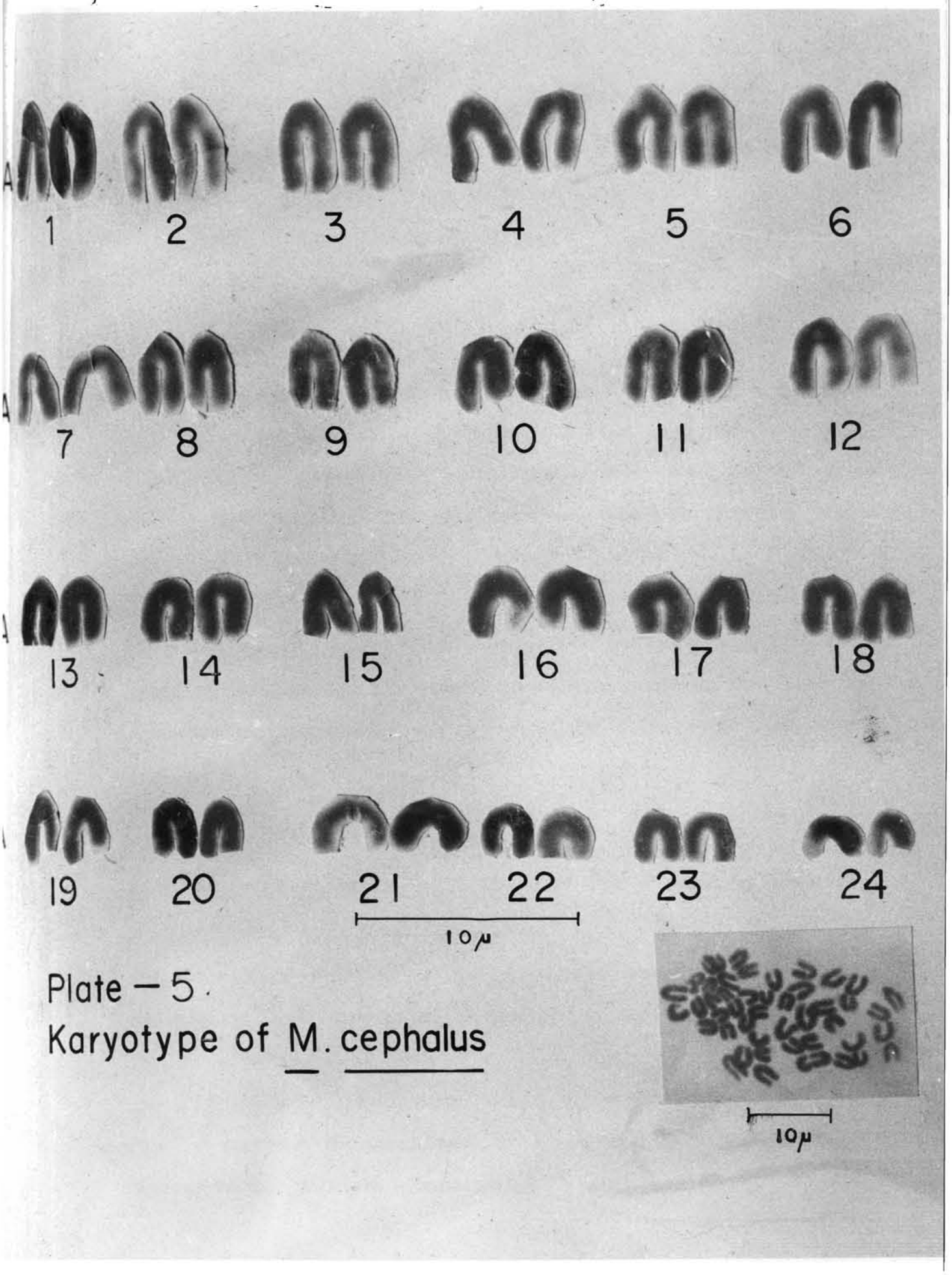


Plate – 5.

Karyotype of M. cephalus

3. BASE LINE VALUES

To study the base line chromosome aberration and micronuclei, L.parsia and M.cephalus were collected from ponds at Narakal and the following findings were obtained.

L.parsia

Chromosome aberrations: In order to study the chromosome aberration, 1083 metaphases were examined. Average 50 metaphase per individual were observed to find out the base line aberration. Aberration per metaphase of this species was found to be 0.0055. Chromosome aberration like gaps were only obtained from the control fish (Table 7).

Micronuclei: In control animal, 5000 cells per individual were examined to find out the occurrence of micronuclei. The base line frequency of micronuclei was found to be 0.0006 per cell (0.06%) (Table 19).

M. cephalus

Chromosome aberrations: The base line chromosome aberrations in M.cephalus are shown in Table 8. In all, 950 metaphase were examined. The rate of aberrations per metaphase was 0.0042, Only aberrations in the form of gaps were observed.

Micronuclei: In control fishes, 5000 cells were scored per fish to find out the occurrence of micronuclei. The occurrence of micronuclei per cell was 0.0004 ((0.04%) (Table 20).

4. TREATMENTS

Five different types of pesticides methyl parathion 50% EC (Metacid 50), phosphamidon 85% SL (Dimecron), dichlorvos 76% EC (Nuvan), monocrotophos 36% SL (Nuvacron), malathion 50% EC) were tried to find their induction of chromosome aberrations, micronuclei formation and behavioural changes in L.parsia and M.cephalus. Standardisation of chromosome preparation has revealed that gill tissue is the most suitable material to prepare chromosome for aberration study. Three doses (lowest-0.01 ppm, middle 0.1 ppm and highest 0.2 ppm) were tried in all pesticide treatments to find out frequency of aberration and occurrence of micronuclei. All treatments were terminated after 96 hrs exposure. Since live animals were required to prepare the chromosome, sublethal concentrations of pesticide were tried.

a) Methyl Parathion 50% EC (Metacid-50)

L.parsia

Chromosome aberrations: About 361 metaphases were examined to find out the chromosome aberration in control fishes. In treated fishes, 202, 225 and 250 metaphases were examined in lowest, middle and highest dose of exposures respectively. Aberration per metaphase in lowest, middle and highest were 0.0346, 0.0666 and

0.1200 respectively against the control value of 0.0055 per metaphase. All doses showed significant increase in the rate of chromosome aberration. Aberration per metaphase was found to increase from lowest treatments to highest dose treatments. The gaps, breaks, fragments, rings, isochromatid gaps, isochromatid breaks and centromeric separations were scored and are presented in Table 9 and Fig.5.

Micronuclei: Table 21 and Fig17 shows the result of induction of micronuclei formation in L.parsia after exposure to methyl parathion. Micronuclei formation increased from the lowest dose of pesticide to the highest dose. The effect of these three doses were found to be significantly higher in experimental fishes than that of control fishes.

M.cephalus

Chromosome aberrations: The rate of chromosome aberration was found to increase from lowest to highest doses. Aberration per metaphase was 0.1033 in highest dose against the control rate of 0.0042 per metaphase. Lowest, middle and highest doses were found to exhibit significant increase in chromosome aberration (Table 14 and Fig.10).

Micronuclei: The frequency of micronuclei formation in erythrocyte cells of M.cephalus are shown in Table 26 and Fig.22. The occurrence of micronuclei was found to increase from lower to highest dose. The number of micronuclei per cell was 0.0060 in lowest dose, 0.0196 in middle dose and 0.0346 in highest dose against the control value of 0.0004 per cell. Occurrences of micronuclei were significantly increased in these three doses.

b) Phosphamidon 85% SL (Dimecron)

L.parsia

Chromosome aberrations: Aberrations per metaphase in lowest, middle and highest dose were 0.0129, 0.0330 and 0.1176 respectively against the control value of 0.0055 per metaphase. The middle and highest dose showed significant increase in the rate of chromosome aberrations. The aberrations were in the form of gaps, breaks, fragments, rings and isochromatid gaps (Table 10 and Fig.6).

Micronuclei: The results of micronuclei formation in fishes which were exposed to phosphamidon for 96 hrs are as following. Micronuclei per cell in lowest, middle and highest doses were 0.0016, 0.0036 and 0.0086 respectively against the control value of 0.0006 per cell. Although the occurrence of micronuclei was increased from lowest to highest dose, significant increase was found to be in the middle and highest dose (Table 22 and Fig. 18).

M.cephalus

Chromosome aberrations: The rate of aberration per metaphase were 0.0158, 0.0514 and 0.1000 respectively against the value of control fishes which was found to be 0.0042 per metaphase. Aberrations per metaphase was significantly increased in all doses of pesticides. Several types of aberrations such as gaps, breaks, bit chromosomes, centromeric separations, etc. were observed. Concentration of pesticide and the increase of chromosome aberration found to be directly proportional (Table 15 and Fig.11).

Micronuclei: Table 27 and Fig.23 shows the result of frequency of micronuclei formation after 96 hrs exposure to phosphamidon. Though the micronuclei formation in lowest, middle and highest doses were found to be higher than that of control fishes, only the highest dose showed statistically significant effect.

c) Dichlorvos 76% EC (Nuvan)

L.parsia

Chromosome aberrations: The rate of chromosome aberrations induced by lowest, middle and highest dose of pesticide were 0.0116, 0.0779 and 0.1125 respectively against the control value of

0.0055 per metaphase. The middle and highest doses in experiment showed significant increase in the rate of chromosome aberration than that of the control fishes. The aberration spectrum included gaps, breaks, isochromatid gaps, isochromatid breaks, fragments and rings (Table 11 and Fig.7).

Micronuclei: The occurrence of micronuclei per cell was 0.0020, 0.0030 and 0.0093 induced by the dose of lowest 0.01 ppm, middle - 0.1 ppm and highest - 0.2 ppm respectively. Only the lowest dose showed insignificant occurrence of micronuclei. The formation of micronuclei was directly proportional to concentration of pesticides (Table 23 and Fig.19).

M.cephalus

Chromosome aberrations: Aberrations per metaphase induced by the three doses lowest 0.01 ppm, middle 0.1 ppm and highest 0.2 ppm) were 0.0096, 0.0210 and 0.0292 respectively against the control value of 0.0042 per mtaphase. Even if the aberration per metaphase was found to increase from lowest to highest dose, a significant increase was noted only in the middle and highest dose. Gaps, breaks, multiple aberration, fragments, etc. were observed in treated fishes. Only one aberration (Gap) was noted in all the 475 metaphase examined from control fishes (Table 16 and Fig.12).

Micronuclei: The frequency of occurrence of micronucleus in three doses (lowest, middle and highest) was found to increase from lowest to highest. The occurrence was increased from 0.0013, 0.0076 and 0.0173 respectively against the value of 0.0004 of control fishes. Significant increase was observed only in middle and highest doses (Table 28 and Fig.24).

d) Monocrotophos 36% SL (Nuvacron)

L.parsia

Chromosome aberrations: The rate of chromosome aberrations were increased from lowest to highest doses of the test chemical. Aberrations per metaphase were 0.0129, 0.0333 and 0.0923 respectively as against the control value of 0.0055 per metaphase. The aberrations per metaphase was significantly higher in middle and highest doses. The doses of test chemical and aberrations in chromosome were found to be direclty proprotional. Gaps, fragments, isochromatic gaps and breaks and centromeric separations were observed in treated fishes (Table 12 and Fig.8).

Micronculei: The occurrence of micronuclei was increased from lowest to highest dose. The frequency of micronuclei per cell was 0.0016, 0.0073 and 0.0180 respectively as against the control value

of 0.0006 per cell. Signiiicant increase was observed only in middle and highest doses (Table 24 and Fig.20).

M.cephalus

Chromosome aberrations: Table 17 and Fig.13 shows the results of chromosome aberration in M.cephalus after 96 hrs exposure to three doses (Lowest-0.01 ppm, middle-0.10 ppm and highest-0.20 ppm) of pesticides. The aberrations were found to increase form lowest to highest doses (0.0128 in lowest, 0.0233 in middle and 0.0436 in highest). Of these, signiiicantly higher aberrations were observed only in middle and highest doses. Gaps, breaks, fragments, centromeric separations ring chromosomes were observed in treated iishes.

Micronuclei: The frequency of micronucleus in M.cephalus exposed to monocrotophos at lowest (0.01 ppm), middle (0.10ppm) and highest (0.20ppm) showed increase in the frequency of micronuclei from lowest to highest dose. Significant increase of micronucleus was obtained in middle and highest doses only. Micronuclei per cell was 0.0016, 0.0080 and 0.0226 respectively as against the control value of 0.0004 per cell (Table 29 and Fig.25).

e) Malathion 50% EC

L.parsia

Chromosome aberrations: Among the pesticides used, malathion induced the least frequency of aberration. The aberration per metaphase was 0.0126, 0.0198 and 0.0585 respectively for lowest to higest dose as against the control value of 0.0055 per metaphase. Significant level of chromosome aberrations was observed only with the highest concentration of pesticides. Gaps, breaks, rings, centromeric separations and fragments were scored out from treated fishes (Table 13 and Fig 9).

Micronuclei: The frequency of micronuclei in L.parsia, after 96 hrs of treatment, showed an increase from lowest to highest dose. Significant level of micronuclei formation was noted only in fishes exposed to the highest dose. The occurrence of micronuclei per cell was 0.0016, 0.0023 and 0.0043 for lowest, medium and highest doses respectively against the value of control of 0.0006 per cell. The results are given in Table 25 and Fig.21.

M.cephalus

Choromosme aberrations: Table 18 and Fig.14 shows result of the analysis of chromosome aberration in M.cephalus exposed to malathion for a period of 96 hrs. The induction of chromosome

aberration in fishes treated with malathion was found to be lower when comapred to those treated with all the other pesticides. Aberrations per metaphase was significantly high only with the highest dose. Aberration per metaphase was 0.0055, 0.0090 and 0.0227 respectively in fishes treated with lowest, middle and highest doses against that of the control (0.0042 per mataphase). Chromosome aberration was found to be in dose response manner in fishes treated with malathion. Significant chromosome aberration was observed only with the highest dose. The type of aberrations examined were gaps, breaks, fragments and rings.

Micronuclei: The formation of micronuclei was found to increase from lowest to the highest dose of malathion. Significant level of micronuclei formation was obtained only in the highest doses. The occurrence of micronuclei per cell was 0.0013, 0.0016 and 0.0060 respectively in lower, middle and highest dose, against that of value of control (0.0004 per cell). The induction of micronuclei was found to increase with increase in dose of the test chemical (Table 30 and Fig.26). Fig. 15 and 16 shows the comparison of the chromosomal aberration induced by the five different pesticide in L.parsia and M.cephalus. All treatments have shown that the level of the frequency of aberration increase with the increase in dose of pesticides. Comparison of frequency of

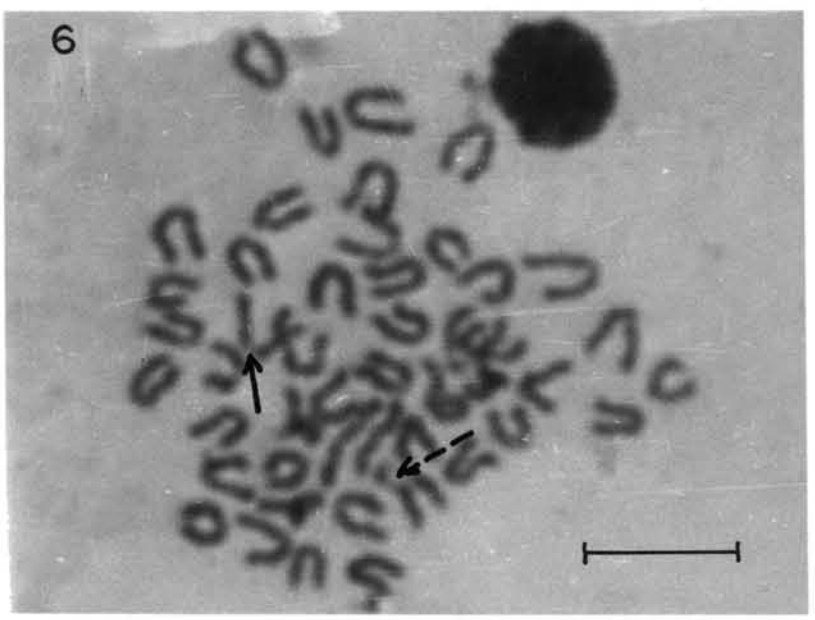
micronucleus in L.parsia and M.cephalus exposed to five different types of pesticides are shown in Fig.27 and 28. Concentration of pesticide highly influenced the formation of micronuclei in these two candidate species. The comparison of chromosome aberrations and occurrence of micronuclei in L.parsia and M.cephalus are given in Table 31 and 32. Table 33 and 34 shows the influence of these pesticide on changes in the behaviour of L.parsia and M.cephalus. Various type of chromosome aberrations were observed in L.parsia and M.cephalus after exposure to earlier mentioned five organophosphorous pesticides (Plates. 6 to 22). Plates 23 to 33 showed nuclear anomali and various shapes of micronuclei attached to nucleus of erythrocytes.

Plate 6. Chromatid break in one of the chromatid (Dotted arrow) and centromeric separation (fission) (smooth arrow) (bar = 10 μ)

Species L.parsia

Plate 7. Chromosome fragment (Dotted arrow) and isochromatid break (Smooth arrow)
(bar = 10 μ)

Species L.parsia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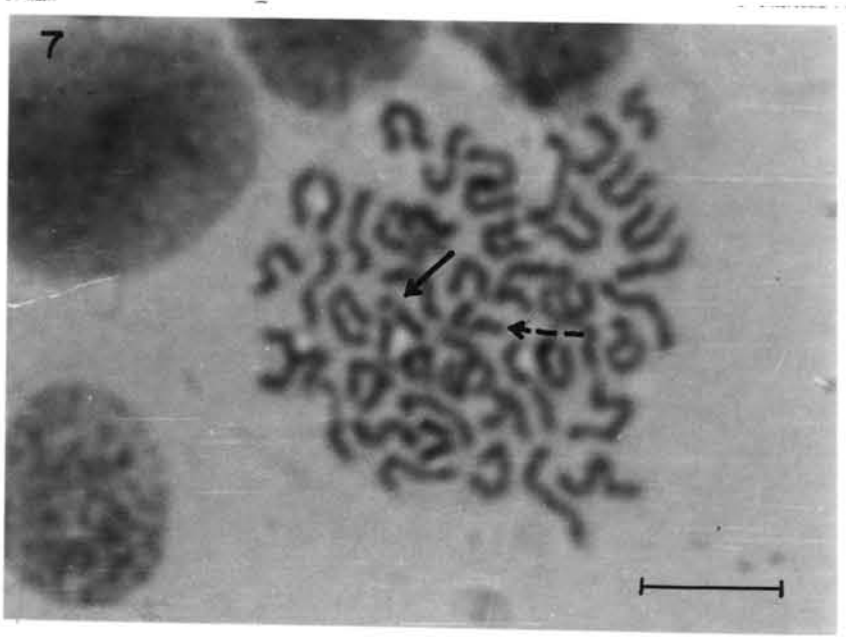
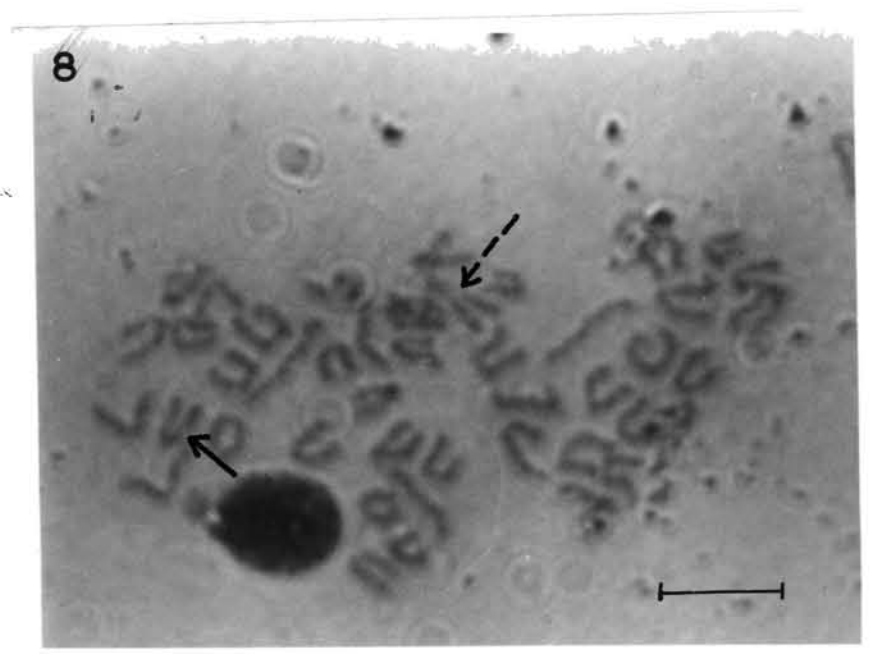

7

Plate 8. Chromatid break (Dotted arrow) and Chromatid gap (smooth arrow)
(bar = 10 μ)

Species - *L. parsia*

Plate 9: Chromatid gap in one of the chromosome and complex rearrangements
(bar = 10 μ)

Species - *L. parsia*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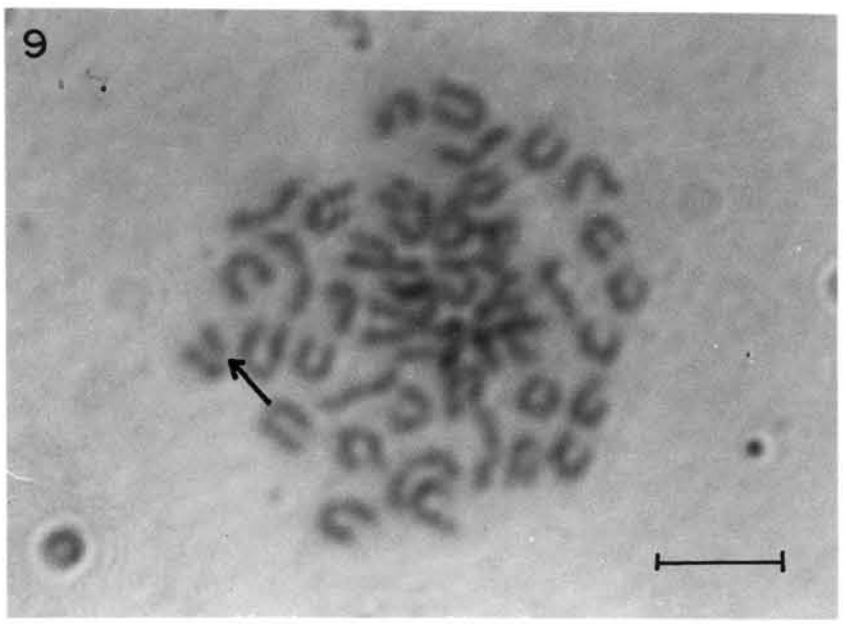
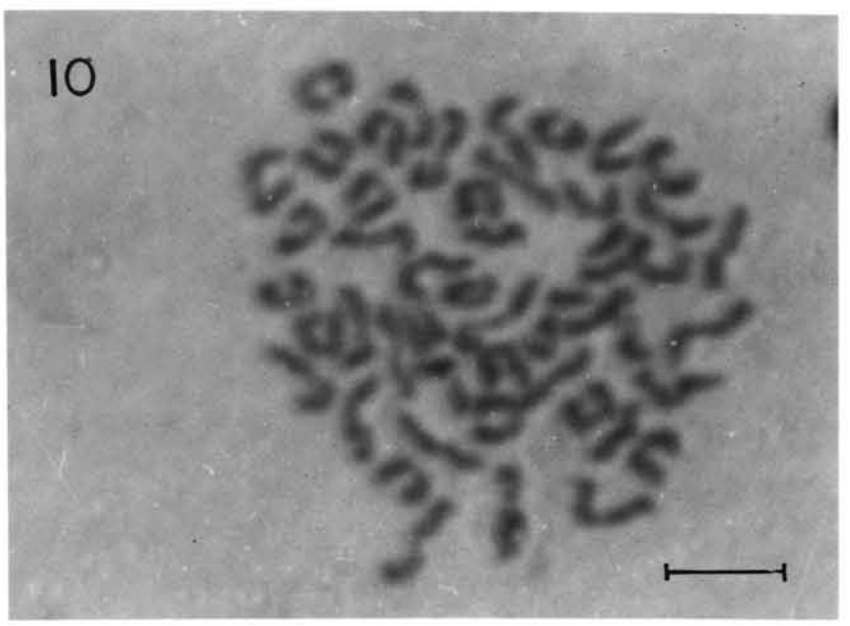

9

Plate 10. Multiple aberrations like gap, breaks, fragments, etc. None of the aberrations are marked
(bar = 10 μ)

Species - L.parsia

Plate 11. Ring chromosomes are marked
(bar = 10 μ)

Species - L.parsia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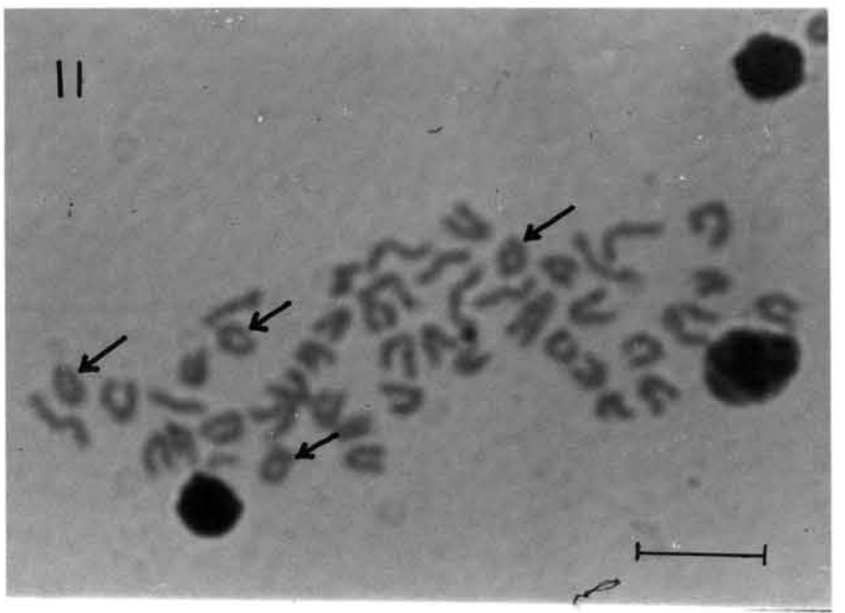
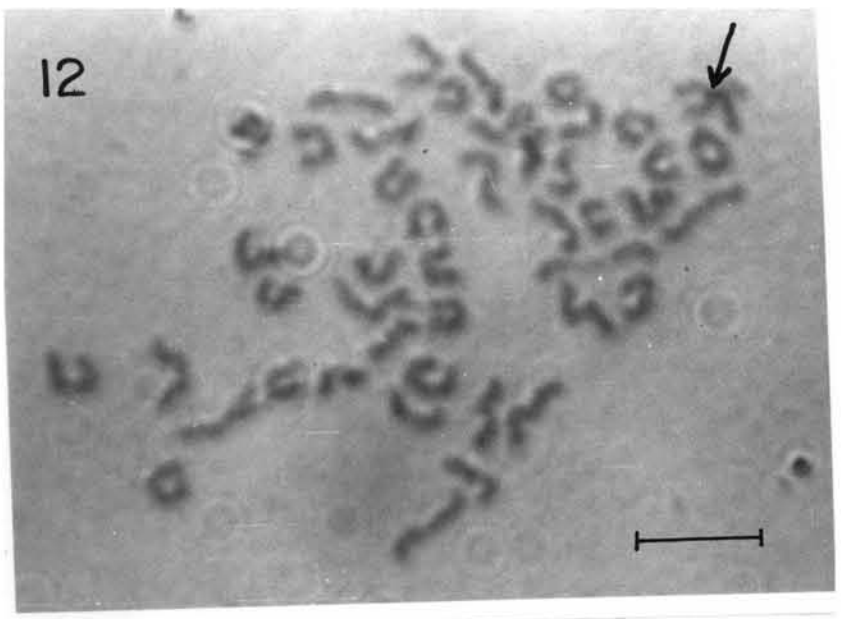

11

Plate 12. Centromeric fusion between chromosomes (bar = 10 μ)

Species - L.parsia

Plate 13. Isochromatid break in one chromosome (bar = 10 μ)

Species - L.parsia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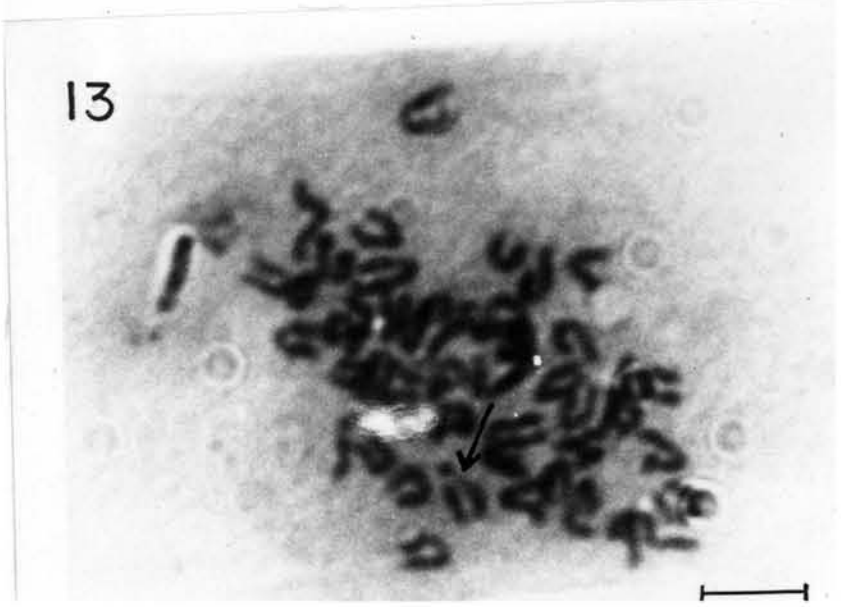

13

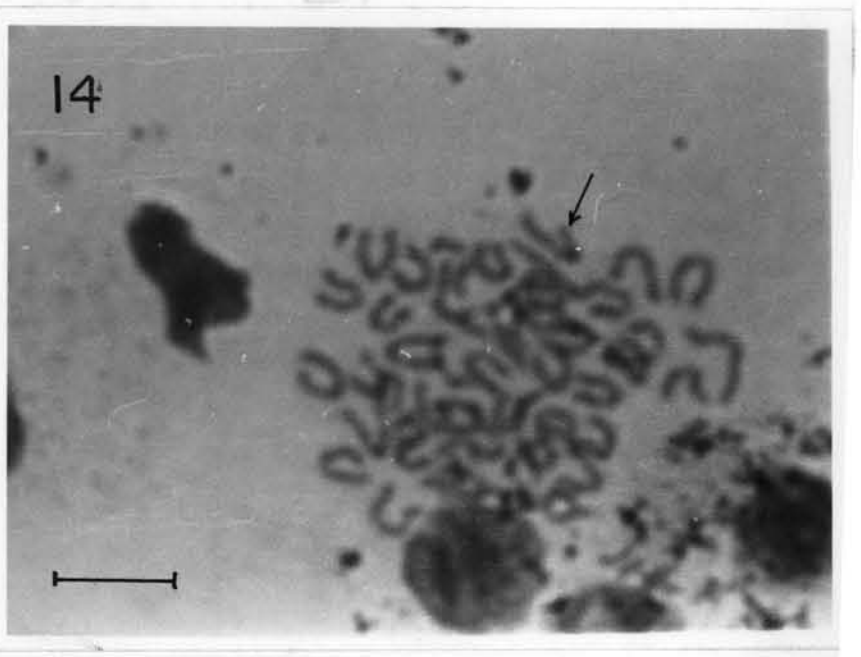
Plate 14. Deletion of distal part of a chromosome,

(bar = 10 μ)

Species - L.parsia

Plate 15. Ring chromosome and some of the chromosome are loosing their entity
(bar = 10 μ)

Species - L.parsia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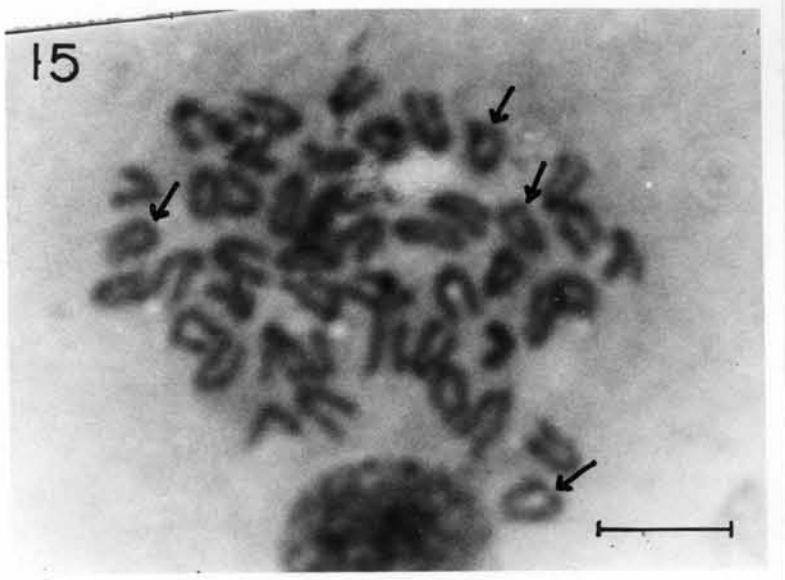
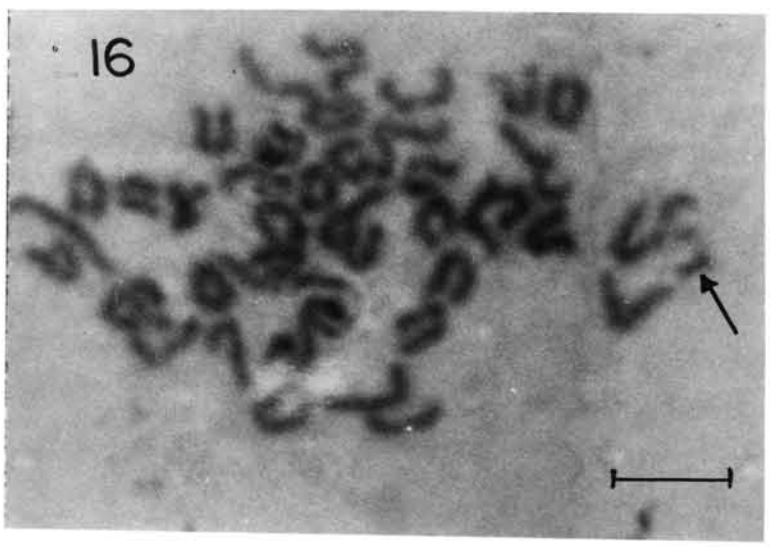

15

Plate 16. Fragment of chromosome
(bar = 10 μ)

Species - L.parsia

Plate 17. Isochromatid break in one of the chromosome
(bar = 10 μ)

Species M.cephalus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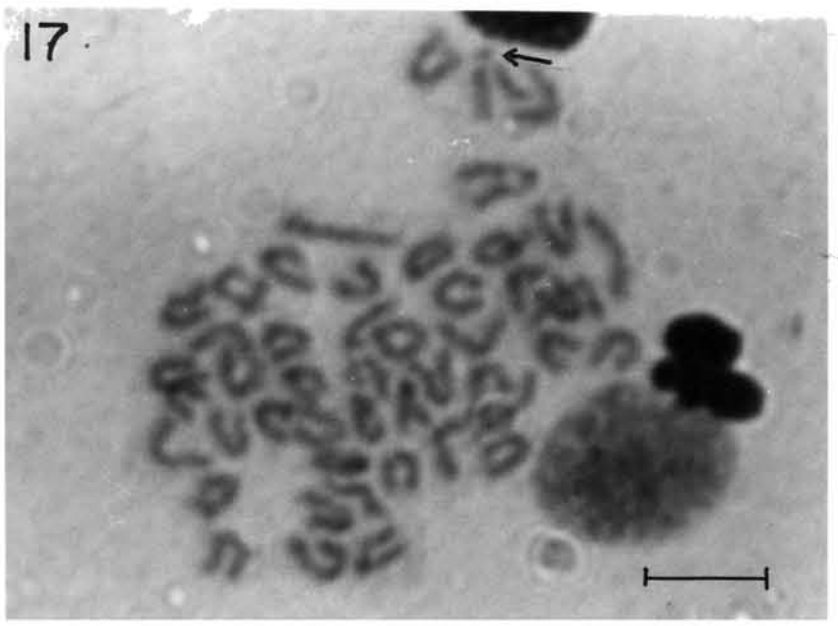
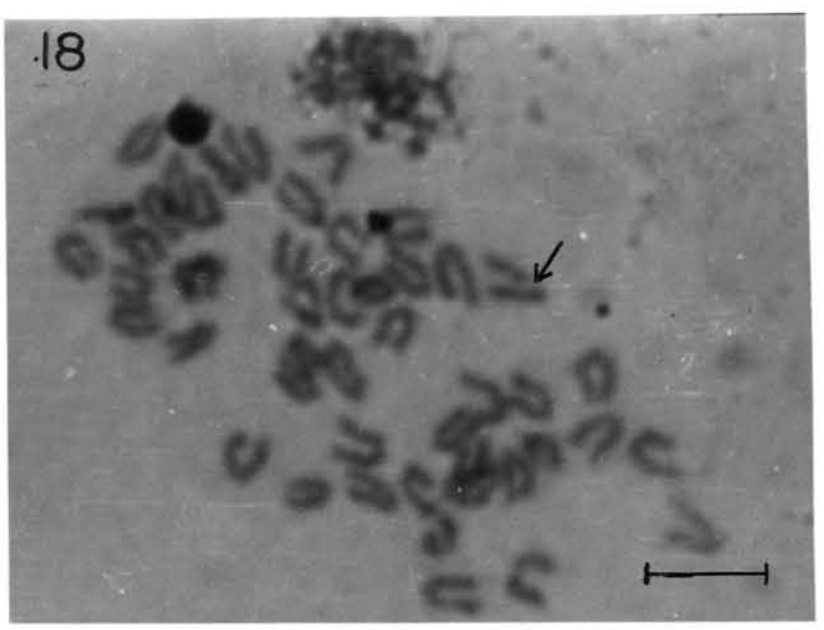

17

Plate 18 . Chromatid break in one of the chromosome
(bar = 10 μ)

Species M.cephalus

Plate 19. Ring chromosome (Dotted arrow), isochromatid gap (smooth arrow) and complex rearrangements.
(bar = 10 μ)

Species M.cephalus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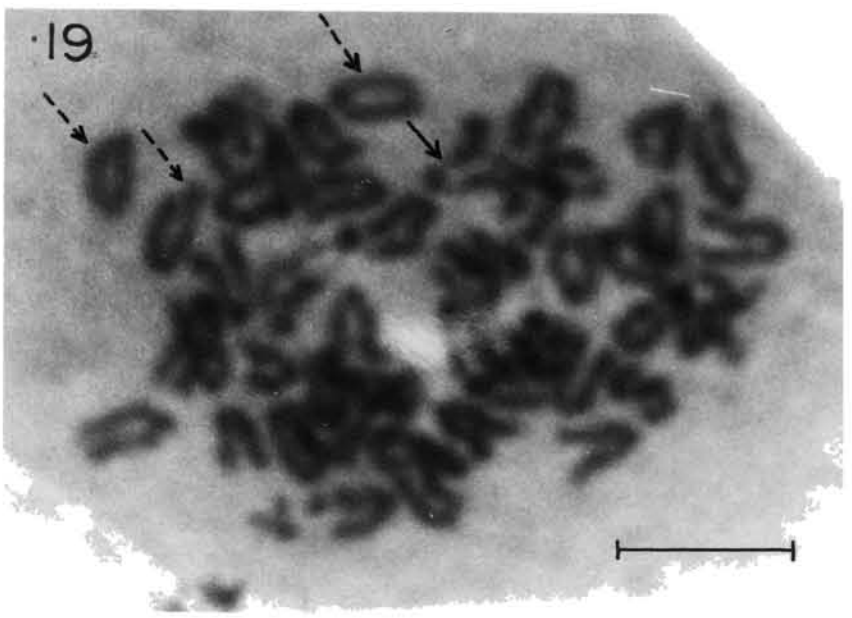
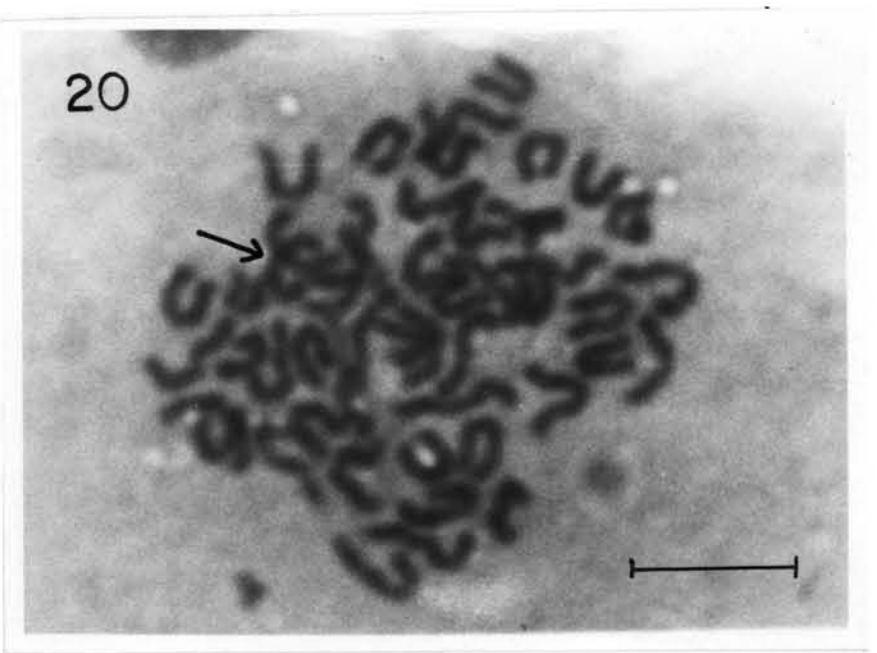

19

Plate 20. Centromeric fusion
(bar = 10 μ)

Species M.cephalus

Plate 21. Multiple aberration like gap, break, fragments. None of the aberrations are marked.
(bar = 10 μ)

Species - M.cephalus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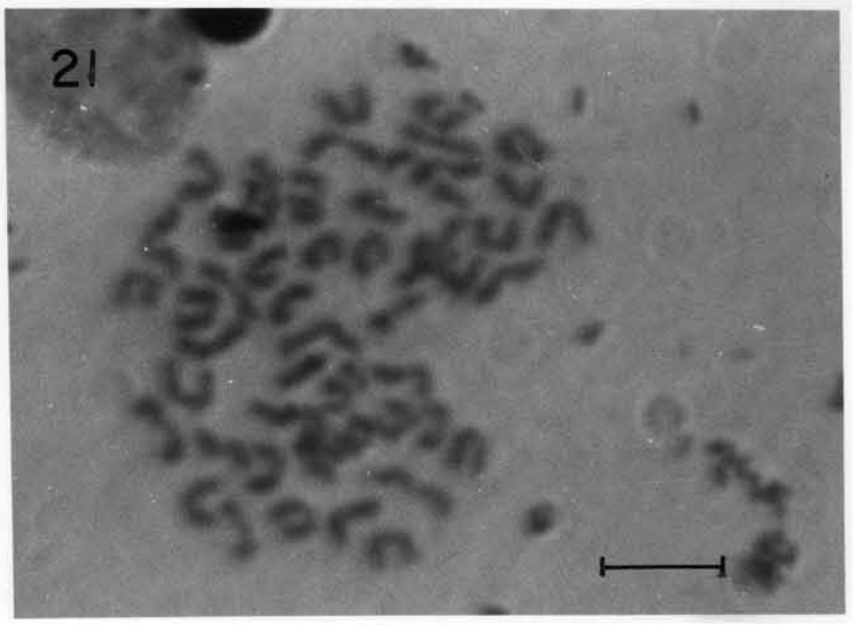
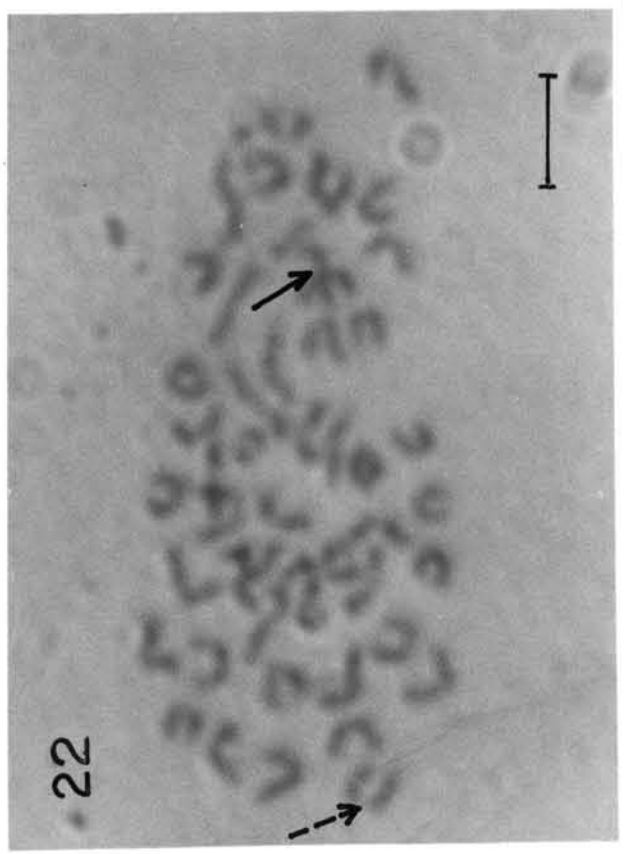

21

Plate 22. Centromeric separation (fission) (Dotted arrow), Centromeric fussion (smooth arrow)
(bar = 10 μ)

Species - M.cephalus

Plate 23. Micronucleated cell, round shape micronucleus is not connected with main nucleus .

Species - L.parsia.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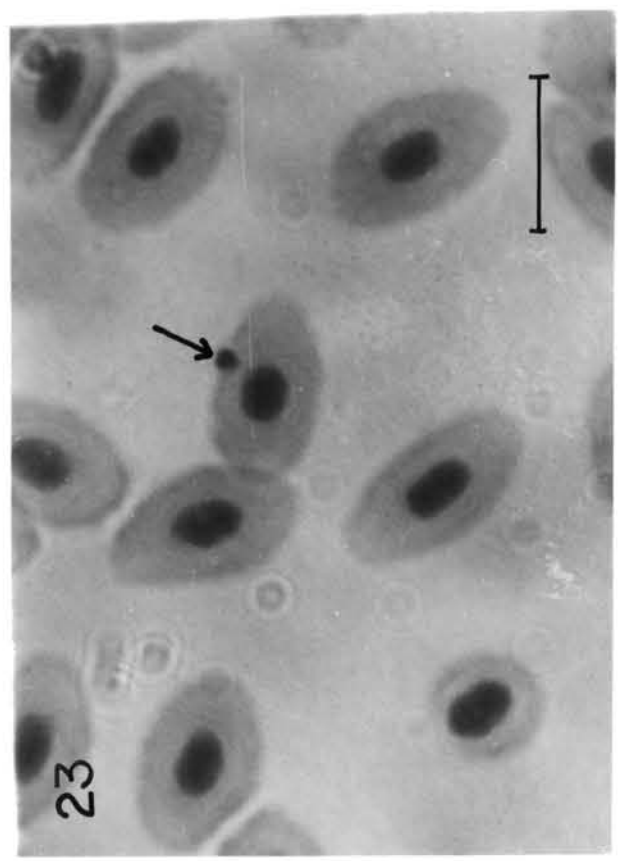
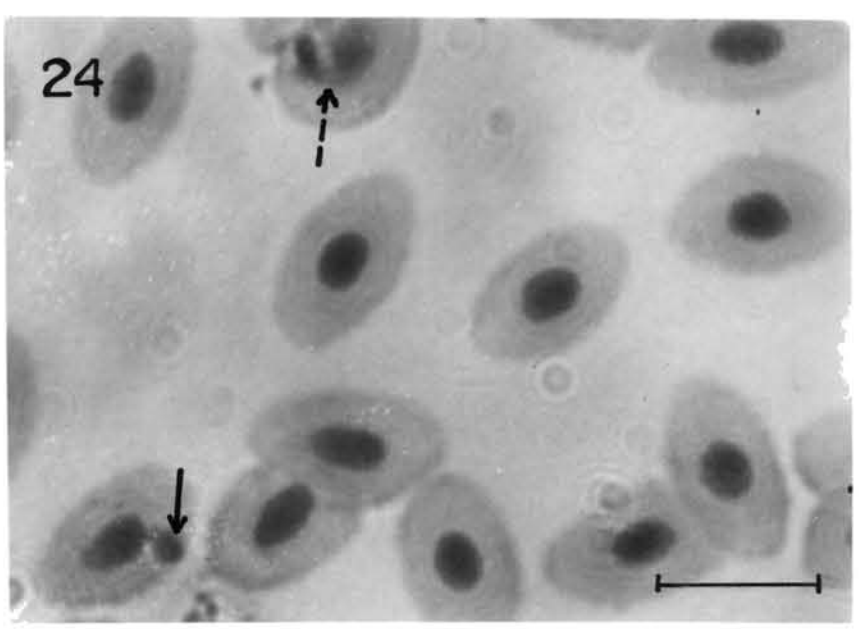

23

Plate 24. Micronucleus is attached to the main nucleus by very thin basophilic strand (smooth arrow) and Lobed erythrocytes (Dotted arrow)

Species - L.parsia.

Plate 25. Micronucleated cells, small micronucleus attached to main nucleus (Dotted arrow) and Micronucleus attached to main nucleus by strand (smooth arrow).

Species - L.parsia.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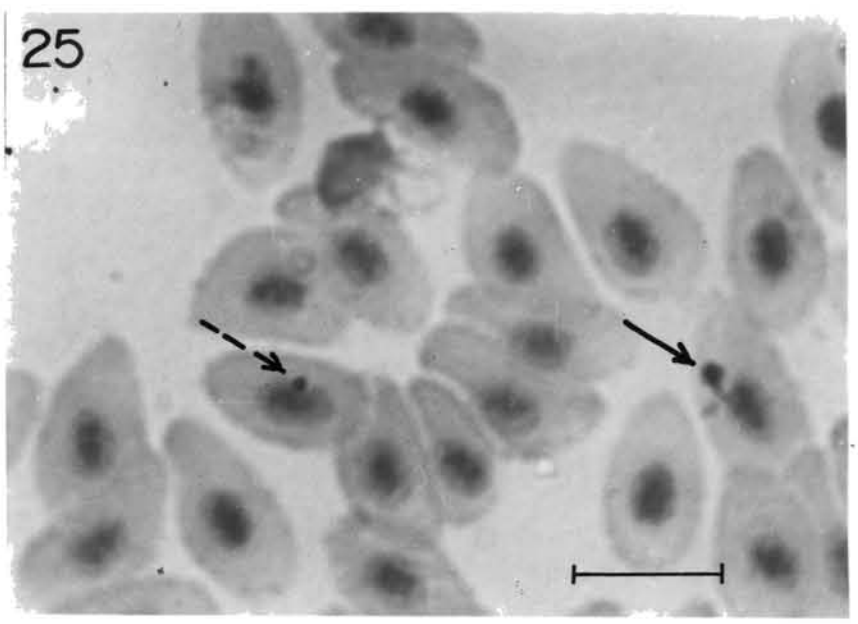
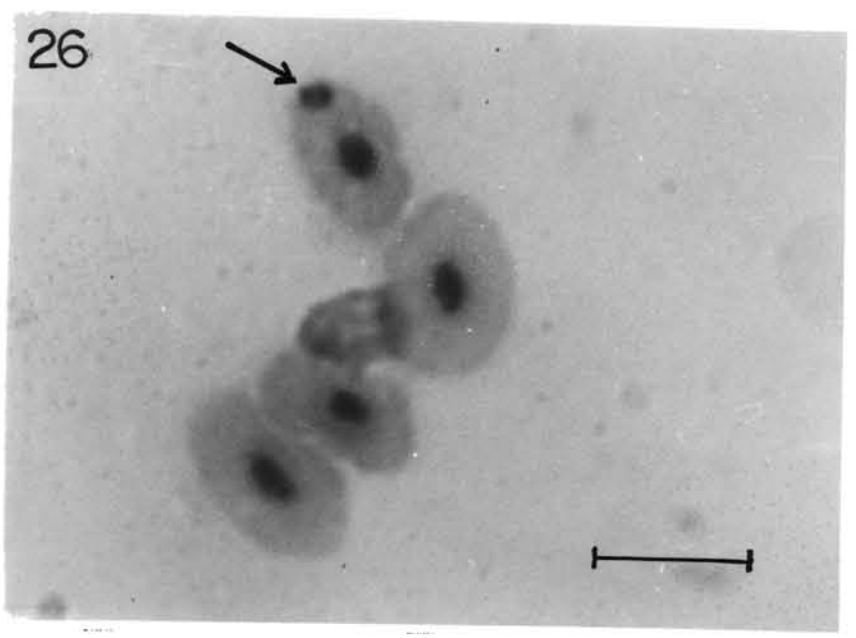

25

Plate 26. Round shape micronucleus attached to peripheral side of erythrocytes

Species - L.parsia.

Plate 27. Structural variation of nucleus in erythrocytes

Species - L.parsia.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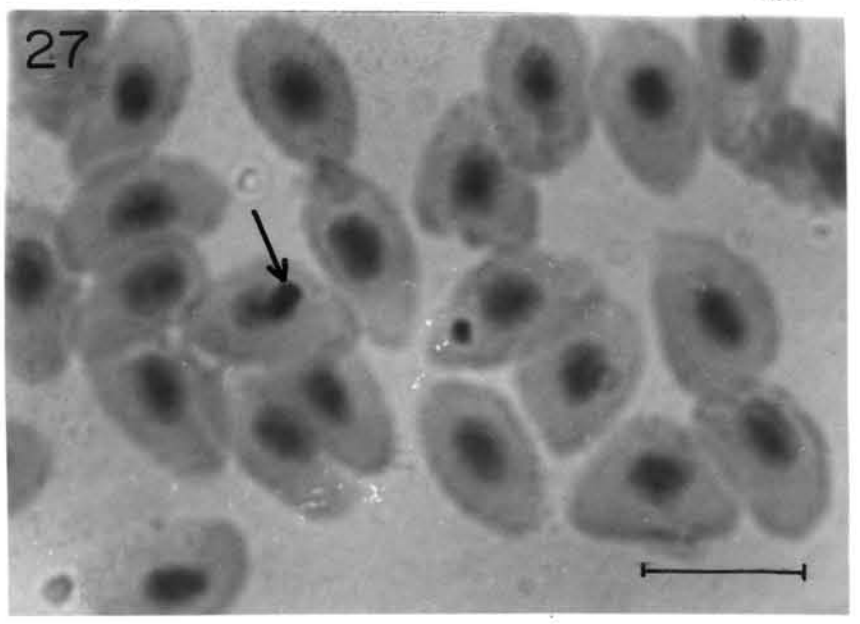
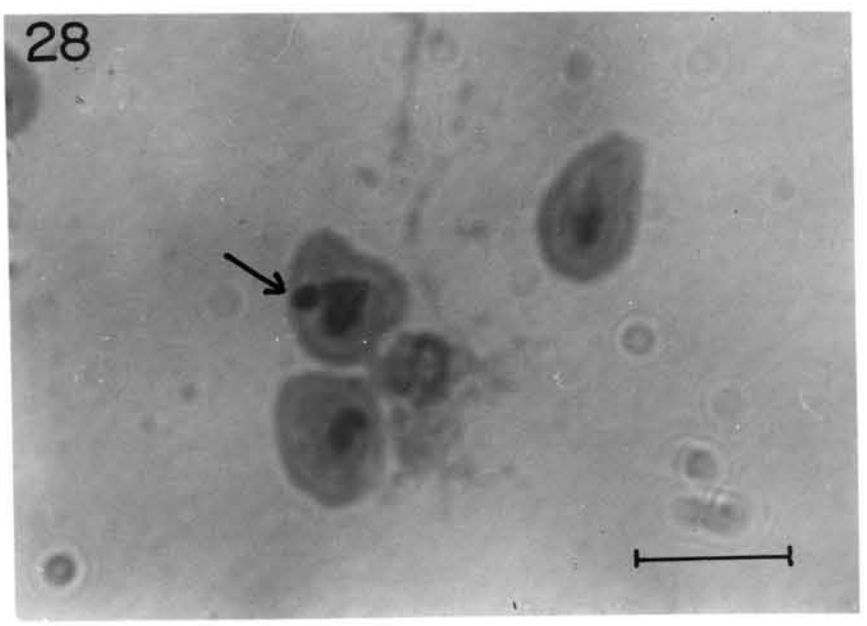

27

Plate 28. Blebbed main nucleus with micronucleus

Species - M.cephalus

Plate 29. Presumed micronucleus attached to peripheral side of erythrocyte

Species - M.cephalus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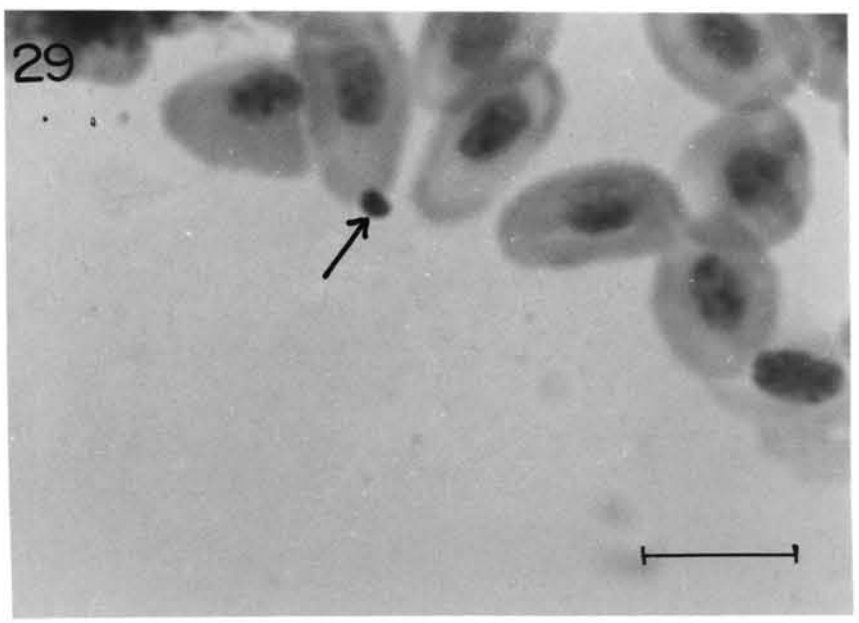
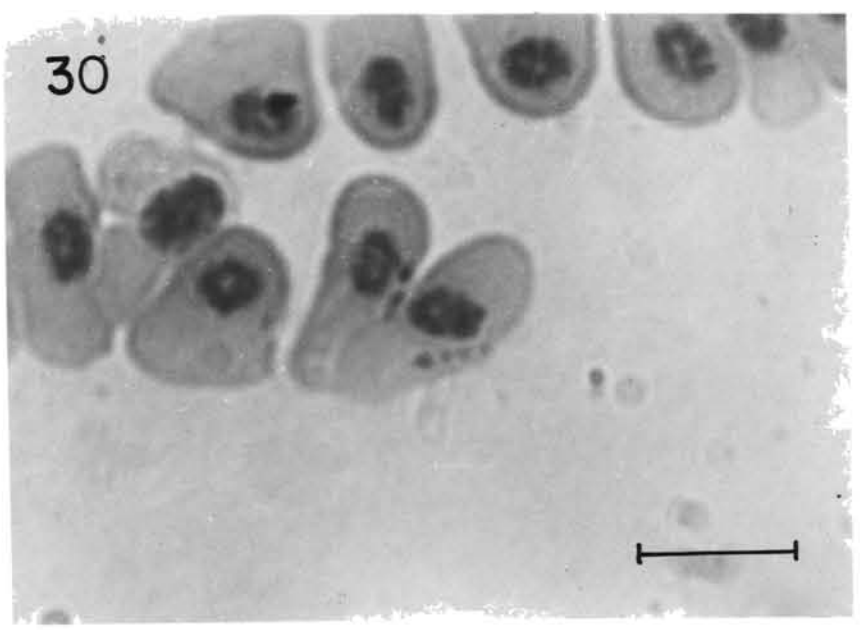

29

Plate 30. Nuclear anomali in erythrocytes

Species - M.cephalus

Plate 31. Round shape nucleus attached to peripheral side of erythrocytes

Species - M.cephalus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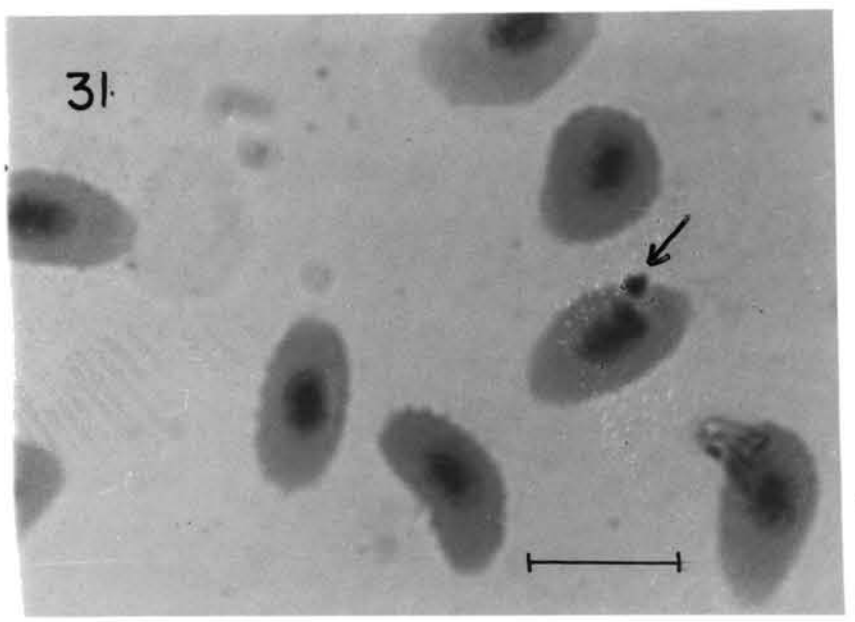
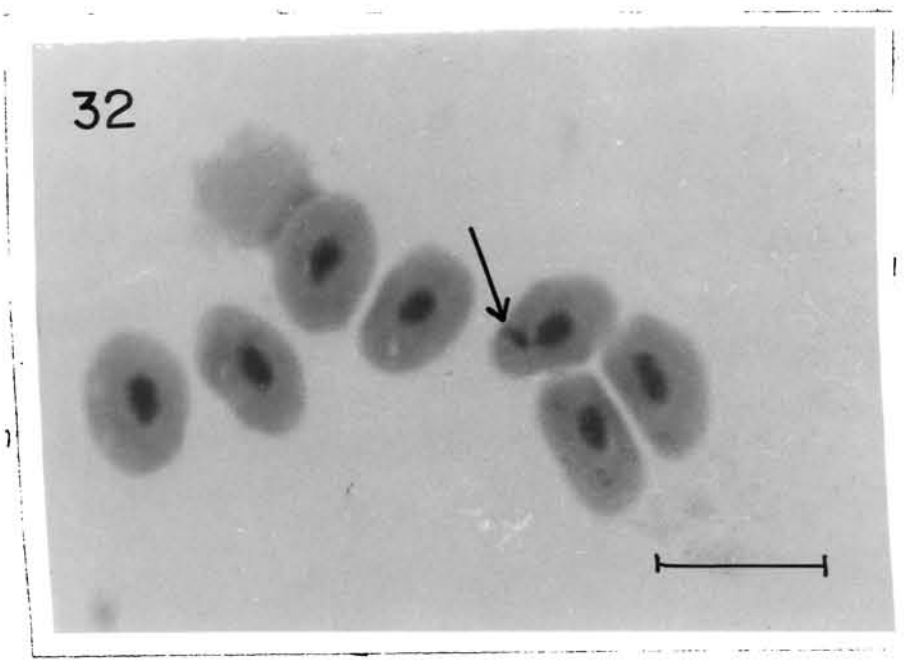

31

Plate 32. Almond shape micronucleus attached to main nucleus by small basophilic strand

Species - M.cephalus

Plate 33. Round shape micronuclei are attached around the main nucleus

Species - M.cephalus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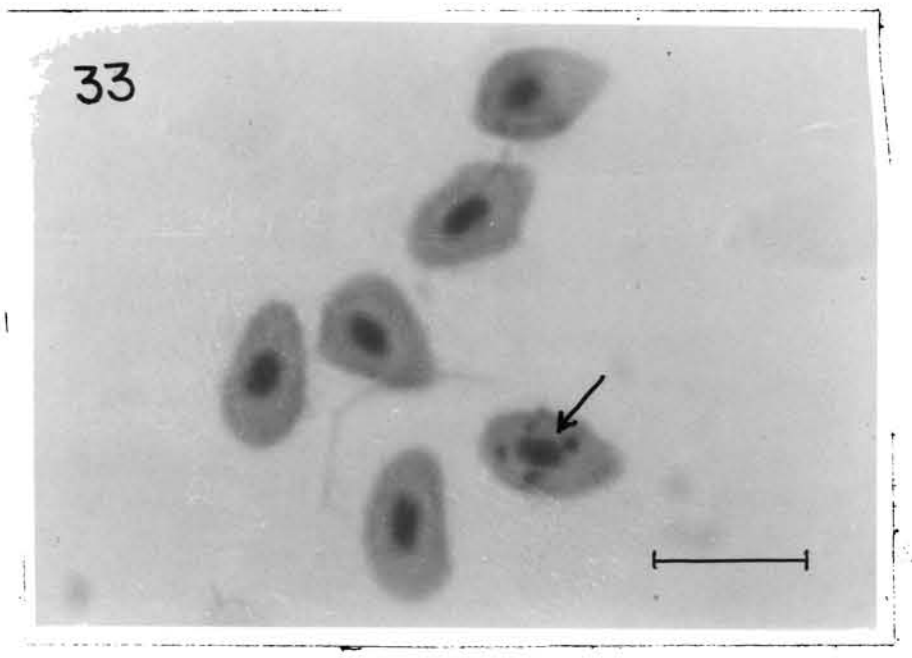
33

Table 1 : Distribution of chromosome numbers of *L.parsia*

Test animals	Chromosome numbers										
	40	41	42	43	44	45	46	47	48	49	50
1	1	1	2	1	2	2	1	2	7	1	
2		1		1		2	2	1	11		
3	1	1	1	2	1	1	1	1	8		
4	1	1	1	2	2	2	2	2	10	1	
5	1	1	1	1		1	3	2	7		
6	1	1	1	1	1	1	1		9	1	
7					2	1			14		
8			1		2		3	1	12		
9				2		3	3		14	1	1
10				2		1		1	13		
11		1	1	1		1		2	11	1	1
12			2	1		2		1	9		
13	1		1	3		2	3	3	6		
14		1		1	2	3	4	1	8		
15	1	1	1	1	3	1		1	12		
16	1	1	1		1	3	2		11		
17	1	1	1		1			3	12		
18	1	1	1	2	1	1	3		10		1

Table 2 : Frequency of chromosome numbers of L.parsia

Chromosome numbers	Frequency
40	10
41	12
42	15
43	21
44	18
45	27
46	28
47	21
48	184
49	5
50	3
Total number of metaphases =	344

Table 3 : Distribution of chromosome numbers of M. cephalus

Test animals	Chromosome numbers									
	41	42	43	44	45	46	47	48	49	50
1	1		1		1	1	1	12	1	
2		1	1	1	1		1	14		
3	1		1	1	1	1		19		
4	1	2	1		1	2	1	21	1	
5		1	1		1	1	1	11	1	1
6		1	2		1	2	1	16		
7	1	1	1		2	1	2	13		
8	1	1	1		1	2	2	14		
9		1	2			1	1	15		1
10	1	1			1	1	2	8	1	
11	1	2	1		2	2	1	10	1	
12		1	1			1	1	15		
13		1	1		1	2	1	9		
14		1	1		1	1	1	16		
15		1			1			12		

Table 4 : Frequency of chromosome numbers of M.cephalus

Chromosome numbers	Frequency
41	7
42	15
43	15
44	2
45	15
46	18
47	16
48	205
49	5
50	2
Total number of metaphases =	300

Table 5 : Chromosome length and relative length - L.parsia

Chromosome Pair No.	Chromosome length (µ) (x ± S.D.)	Relative length (%)	Chromosome Type
1	4.800 ± 0.173	5.332	A
2	4.725 ± 0.144	5.249	A
3	4.700 ± 0.058	5.220	A
4	4.625 ± 0.029	5.137	A
5	4.550 ± 0.058	5.054	A
6	4.500 ± 0.058	4.999	A
7	4.375 ± 0.087	4.859	A
8	4.325 ± 0.144	4.804	A
9	4.250 ± 0.058	4.721	A
10	3.925 ± 0.029	4.359	A
11	3.850 ± 0.058	4.277	A
12	3.800 ± 0.058	4.221	A
13	3.725 ± 0.087	4.138	A
14	3.675 ± 0.029	4.082	A
15	3.525 ± 0.087	3.916	A
16	3.475 ± 0.029	3.860	A
17	3.450 ± 0.058	3.832	A
18	3.375 ± 0.029	3.749	A
19	3.075 ± 0.087	3.416	A
20	3.000 ± 0.058	3.332	A
21	2.875 ± 0.029	3.194	A
22	2.750 ± 0.058	3.055	A
23	2.550 ± 0.058	2.833	A
24	2.125 ± 0.029	2.361	A

Total chromosome length (n) = 90.025 µ

Table 6 : Chromosome length and relative length - M.cephalus

Chromosome Pair No.	Chromosome length (μ) ($\bar{x}$ ± S.D.)	Relative length (%)	Chromosome Type
1	5.5280 ± 0.161	6.402	A
2	5.1675 ± 0.064	5.985	A
3	4.8335 ± 0.192	5.598	A
4	4.1950 ± 0.096	4.858	A
5.	4.1395 ± 0.032	4.794	A
6	4.0560 ± 0.065	4.694	A
7	4.0280 ± 0.032	4.665	A
8	3.9445 ± 0.064	4.568	A
9.	3.7785 ± 0.064	4.376	A
10	3.6395 ± 0.032	4.215	A
11	3.6115 ± 0.064	4.183	A
12	3.5840 ± 0.032	4.150	A
13	3.5560 ± 0.128	4.118	A
14	3.5005 ± 0.064	4.054	A
15	3.2785 ± 0.064	3.797	A
16	3.2505 ± 0.032	3.765	A
17.	3.1395 ± 0.032	3.636	A
18	3.0280 ± 0.032	3.507	A
19	3.0005 ± 0.064	3.475	A
20	2.8615 ± 0.032	3.314	A
21	2.8060 ± 0.096	3.249	A
22	2.6395 ± 0.096	3.057	A
23	2.5285 ± 0.096	2.928	A
24.	2.2505 ± 0.032	2.606	A

Total chromosome length (n) = 86.345 μ

Table 7 : Base line chromosome aberrations in L.parsia

Code of animals	No.of metaphase examined	No.of aberrations*	Aberration per metaphase
A	56	0	0.0000
B	69	0	0.0000
C	79	1	0.0127
D	102	2	0.0196
E	48	0	0.0000
F	52	0	0.0000
G	40	0	0.0000
H	44	1	0.0227
I	60	0	0.0000
J	72	0	0.0000
K	50	0	0.0000
L	81	1	0.0123
M	28	0	0.0000
N	32	0	0.0000
O	48	0	0.0000
P	52	0	0.0000
Q	61	0	0.0000
R	32	0	0.0000
S	23	1	0.0435
T	54	0	0.0000
Total 20	1083	6	0.0055 ± 0.0022
* gap			($\bar{x}$ ± S.E.)

Table 8 : Base line chromosome aberrations in M.cephalus

Code of animals	No.of metaphase examined	No. of aberrations *	Aberration per metaphase
A	48	1	0.0208
B	32	0	0.0000
C	50	0	0.0000
D	58	1	0.0172
E	63	0	0.0000
F	71	0	0.0000
G	49	0	0.0000
H	40	0	0.0000
I	56	0	0.0000
J	55	1	0.0181
K	60	0	0.0000
L	71	0	0.0000
M	57	0	0.0000
N	44	0	0.0000
O	51	1	0.0196
P	43	0	0.0000
Q	44	0	0.0000
R	58	0	0.0000
Total 18	950	4	0.0042 ± 0.0020
* gap			($\bar{x}$ ± S.E.)

Table 9 : Analysis of chromosome aberrations in L.parsia exposed to Methyl Parathion (Metacid).

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	361	2	0.0055 ± 0.0038
0.01	202	7	0.0346 ± 0.0128 **
0.1	225	15	0.0666 ± 0.0166 **
0.2	250	30	0.1200 ± 0.0205 **

** Significant ($Z \geqslant 1.96$)

* gap, break, fragment, ring, isochromatid break, isochromatid gap, centromeric separation

Table 10 : Analysis of chromosome aberrations in L.parsia exposed to Phosphamidon (Dimecron)

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	361	2	0.0055 ± 0.0038
0.01	232	3	0.0129 ± 0.0074
0.1	242	8	0.0330 ± 0.0114 **
0.2	102	12	0.1176 ± 0.0318 **

** Significant ($Z \geqslant 1.96$)

* gap, break, fragment, ring, isochromatid gap

Table 11 : Analysis of chromosome aberrations in L.parsia exposed to Dichlorvos (Nuvan)

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	361	2	0.0055 ± 0.0038
0.01	172	2	0.0116 ± 0.0081
0.1	154	12	0.0779 ± 0.0215 **
0.2	160	18	0.1125 ± 0.0249 **

** Significant ($Z \geqslant 1.96$)

* gap, break, isochromatid gap, isochromatid break, fragment, ring.

Table 12 : Analysis of chromosome aberrations in L.parsia exposed to Monocrotophos (Nuvacron)

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	361	2	0.0055 ± 0.0038
0.01	232	3	0.0129 ± 0.0074
0.1	210	7	0.0333 ± 0.0123 **
0.2	195	18	0.0923 ± 0.0207 **

** Significant ($Z \geqslant 1.96$)

* gap, fragment, isochromatid gap and break, centromeric separation.

Table 13 : Analysis of chromosome aberrations in L.parsia exposed to Malathion.

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	361	2	0.0055 ± 0.0038
0.01	238	3	0.0126 ± 0.0072
0.1	202	4	0.0198 ± 0.0098
0.2	188	11	0.0585 ± 0.0171 **

** Significant ($Z \geqslant 1.96$)

* gap, break, ring, centromeric separation, chromosome fragment.

Table 14 : Analysis of chromosome aberrations in M.cephalus exposed to Methyl Parathion (Metacid).

Dose (ppm)	No.of metaphase examined	Total number of of aberrations*	Aberration per metaphase ± S.E.
Control	475	2	0.0042 ± 0.0029
0.01	212	8	0.0377 ± 0.0130 **
0.1	252	19	0.0753 ± 0.0166 **
0.2	271	28	0.1033 ± 0.0184 **

** Significant ($Z \geqslant 1.96$)

* gap, fragment, break, ring, isochromatid gap, multiple aberration.

Table 15 : Analysis of chromosome aberrations in M.cephalus exposed to Phosphamidon (Dimecron).

Dose (ppm)	No.of metaphase examined	Total number of aberrations *	Aberration per metaphase ± S.E.
Control	475	2	0.0042 ± 0.0029
0.01	252	4	0.0158 ± 0.0078
0.1	214	11	0.0514 ± 0.0151 **
0.2	200	20	0.1000 ± 0.0212 **

** Significant ($Z \geqslant 1.96$)

* gap, multiple aberration, break, bit chromosome, centromeric separation.

Table 16 : Analysis of chromosome aberrations in M.cephalus exposed to Dichlorvos (Nuvan)

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	475	2	0.0042 ± 0.0029
0.01	312	3	0.0096 ± 0.0055
0.1	380	8	0.0210 ± 0.0073 **
0.2	342	10	0.0292 ± 0.0091 **

** Significant ($Z \geqslant 1.96$)

* gap, break, multiple aberration, fragment.

Table 17 : Analysis of chromosome aberrations in M.cephalus exposed to Monocrotophos (Nuvacron).

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	475	2	0.0042 ± 0.0029
0.01	312	4	0.0128 ± 0.0063
0.1	342	8	0.0233 ± 0.0081 **
0.2	344	15	0.0436 ± 0.0110 **

** Significant ($Z \geqslant 1.96$)

* gap, break, fragment, centromeric separation, ring chromosome

Table 18 : Analysis of chromosome aberrations in M.cephalus exposed to Malathion.

Dose (ppm)	No.of metaphase examined	Total number of aberrations*	Aberration per metaphase ± S.E.
Control	475	2	0.0042 ± 0.0029
0.01	358	2	0.0055 ± 0.0039
0.1	443	4	0.0090 ± 0.0044
0.2	440	10	0.0227 ± 0.0071 **

** Significant ($Z \geqslant 1.96$)

* gap, break, fragment, ring.

Table 19 : Base line frequency of micronucleus in L.parsia

Code of animals	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell
A	5000	5	0.10	0.0010
B	5000	4	0.08	0.0008
C	5000	5	0.10	0.0010
D	5000	7	0.14	0.0014
E	5000	2	0.04	0.0004
F	5000	4	0.08	0.0008
G	5000	0	0.00	0.0000
H	5000	3	0.06	0.0006
I	5000	5	0.10	0.0010
J	5000	3	0.06	0.0006
K	5000	4	0.08	0.0008
L	5000	2	0.04	0.0004
M	5000	0	0.00	0.0000
N	5000	1	0.02	0.0002
O	5000	4	0.08	0.0008
Total 15	75000	49	0.06	0.0006 ± 0.00008 ($\bar{x}$ ± S.E.)

Table 20: Base line frequency of micronucleus in M.cephalus

Code of animals	No.of cells scored	Total number of micronuceli	Percentage of micronuclei	Micronuclei per cell
A	5000	3	0.06	0.0006
B	5000	4	0.08	0.0008
C	5000	1	0.02	0.0002
D	5000	0	0.00	0.0000
E	5000	0	0.00	0.0000
F	5000	4	0.08	0.0008
G	5000	5	0.10	0.0001
H	5000	0	0.00	0.0000
I	5000	3	0.06	0.0006
J	5000	4	0.08	0.0008
K	5000	4	0.08	0.0008
L	5000	1	0.02	0.0002
M	5000	0	0.00	0.0000
N	5000	0	0.00	0.0000
O	5000	1	0.02	0.0002
Total 15	75000	26	0.04	0.0004 ± 0.00007 (x ± S.E.)

Table 21 : Frequency of micronucleus in L.parsia exposed to Methyl Parathion (Metacid)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	3	0.06	0.0006 ± 0.0003
0.01	3000	14	0.46	0.0046 ± 0.0012 **
0.1	3000	42	1.40	0.0140 ± 0.0021 **
0.2	3000	91	3.03	0.0303 ± 0.0031 **

** significant ($Z \geqslant 1.96$)

Table 22 : Frequency of micronucleus in L.parsia exposed to Phosphamidon (Dimecron)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	3	0.06	0.0006 ± 0.0003
0.01	3000	5	0.16	0.0016 ± 0.0007
0.1	3000	11	0.36	0.0036 ± 0.0010 **
0.2	3000	26	0.86	0.0086 ± 0.0016 **

** Significant ($Z \geqslant 1.96$)

Table 23 : Frequency of micronucleus in L.parsia exposed to Dichlorvos (Nuvan)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	3	0.06	0.0006 ± 0.0003
0.01	3000	6	0.20	0.0020 ± 0.0008
0.1	3000	9	0.30	0.0030 ± 0.0009 **
0.2	3000	28	0.93	0.0093 ± 0.0017 **

** Significant ($Z \geqslant 1.96$)

Table 24 : Frequency of micronucleus in L.parsia exposed to Monocrotophos (Nuvacron)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	3	0.06	0.0006 ± 0.0003
0.01	3000	5	0.16	0.0016 ± 0.0007
0.1	3000	22	0.73	0.0073 ± 0.0015 **
0.2	3000	54	1.80	0.0180 ± 0.0024 **

** Significant ($Z \geqslant 1.96$)

Table 25 : Frequency of micronucleus in L.parsia exposed to Malathion

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	3	0.06	0.0006 ± 0.0003
0.01	3000	5	0.16	0.0016 ± 0.0007
0.1	3000	7	0.23	0.0023 ± 0.0008
0.2	3000	13	0.43	0.0043 ± 0.0011 **

** Significant ($Z \geqslant 1.96$)

Table 26 : Frequency of micronucleus in M.cephalus exposed to Methyl Parathion (Metacid)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	2	0.04	0.0004 ± 0.0002
0.01	3000	18	0.60	0.0060 ± 0.0014 **
0.1	3000	59	1.96	0.0196 ± 0.0025 **
0.2	3000	104	3.46	0.0346 ± 0.0033 **

** Significant ($Z \geqslant 1.96$)

Table 27 : Frequency of micronucleus in M.cephalus exposed to Phosphamidon (Dimecron)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	2	0.04	0.0004 ± 0.0002
0.01	3000	5	0.16	0.0016 ± 0.0007
0.1	3000	8	0.26	0.0026 ± 0.0009**
0.2	3000	36	1.20	0.0120 ± 0.0019 **

** Significant ($Z \geqslant 1.96$)

Table 28: Frequency of micronucleus in M.cephalus exposed to Dichlorvos (Nuvan)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	2	0.04	0.0004 ± 0.0002
0.01	3000	4	0.13	0.0013 ± 0.0006
0.1	3000	23	0.76	0.0076 ± 0.0015 **
0.2	3000	52	1.73	0.0173 ± 0.0023 **

Significant ($Z \geqslant 1.96$)

Table 29 : Frequency of micronucleus in M.cephalus exposed to Monocrotophos (Nuvacron)

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	2	0.04	0.0004 ± 0.0002
0.01	3000	5	0.16	0.0016 ± 0.0007
0.1	3000	24	0.80	0.0080 ± 0.0016 **
0.2	3000	68	2.26	0.0226 ± 0.0027 **

** Significant ($Z \geqslant 1.96$)

Table 30 : Frequency of micronucleus in M.cephalus exposed to Malathion

Dose (ppm)	No.of cells scored	Total number of micronuclei	Percentage of micronuclei	Micronuclei per cell ± S.E.
Control	5000	2	0.04	0.0004 ± 0.0002
0.01	3000	4	0.13	0.0013 ± 0.0006
0.1	3000	5	0.16	0.0016 ± 0.0007
0.2	3000	18	0.60	0.0060 ± 0.0014 **

** Significant ($Z \geqslant 1.96$)

Table 31 : Comparison of chromosome aberrations and frequency of Micronucleus in L.parsia exposed to various pesticides

Name of pesticides	Chromosome aberrations Dose (ppm)			Micronuclei Dose (ppm)		
	0.01	0.1	0.2	0.01	0.1	0.2
Methyl Parathion	+	+	+	+	+	+
Phosphamidon	-	+	+	-	+	+
Dichlorvos	-	+	+	-	+	+
Monocrotophos	-	+	+	-	+	+
Malathion	-	-	+	-	-	+

\+ : Significant at 5% level ($Z \geqslant 1.96$)

\- : Insignificant at 5% level ($Z \geqslant 1.96$)

Table 32 : Comparison of chromosome aberrations and frequency of micronucleus in M.cephalus exposed to various pesticides

Name of pesticides	Chromosome aberrations Dose (ppm)			Micronuclei Dose (ppm)		
	0.01	0.1	0.2	0.01	0.1	0.2
Methyl Parathion	+	+	+	+	+	+
Phosphamidon	-	+	+	-	+	+
Dichlorvos	-	+	+	-	+	+
Monocrotophos	-	+	+	-	+	+
Malathion	-	-	+	-	-	+

\+ : Significant at 5% level ($Z \geqslant 1.96$)

\- : Insignificant at 5% level ($Z \geqslant 1.96$)

Table 33 :Behavioural changes of L.parsia after 96 hrs exposure to pesticides

Name of pesticides	Reaction of Fishes		
	0.01 ppm	0.1 ppm	0.2 ppm
Methyl Parathion	Vigorous escapism, slow gasping at the initial 1 hr, later escapism behaviour subsided then slow swi mming at 72 hrs, resting , opercular movement decreased, jerky movement on prodding.	Violent movement at initial 3 hrs, resting and gasping, movement on prodding, at the end of 72 hrs, loss of equilibrium at 90th hrs.	Jumping, whirling movement, restless in the initial 5 hrs then gasping on opercular movement increased, loss of equilibrium, lying on lateral side, and swimming up side down intermittently at the 96th hrs of experiment.
Phosphamidon	Fast whirling movement at the initial 1 hr of treatment then resting and gulping at the bottom of the tank, afterwards came to normal state.	Same as in 0.01 ppm upto 48 hrs, but later showed sluggish and jerking movement, finally resting at the bottom of the tank.	Jumping and escaping behaviour, slow gasping& swimming after 24 hrs treatments, loss of equilibrium at 90th hrs.
Dichlorvos	Vigorous whirling movement upto 1 hr of treatment, resting, slow gasping, opercular movement decreased upto 24 hrs. then showed normal behaviour.	Vigorous whirling and jumping movement upto 10 hrs, then slow swimming in the surface and resting.	Violent movement in the surface of water upto initial 3 hrs then resting and gasping with increased opercular movement, jerky movement on prodding.

Monocrotophos -	Whirling movement on surface of water upto 30 min., resting intermittently, gulping, slow swimming upto 24 hrs, then fishes showed normal behaviour.	Slow swimming, fast gasping, opercular movement increased, lying on the side and gulping at the end of experiment.	All fishes come to surface within 1 hr, irregular vertical revolving movement, opercular movement increased slowly, lost their balance after 72 hrs, feeble opercular movement at the 96th hrs of experiment.
Malathion	Slow swimming upto 24 hrs of treatment then resting and normal behaviour showed at the end of the treatment.	Same as in the case of 0.01 ppm.	Violent jumping behaviour at the initial 1 hr treatment, later on fishes showed slow swimming and lying on the bottom, violent movement on prodding, feeble opercular movement.

Table 34 : Behavioural changes of M.cephalus after 96 hrs exposure to pesticides.

Name of Pesticides	Reaction of Fishes		
	0.01 ppm	0.1 ppm	0.2 ppm
Methyl Parathion	Fast swimming, vertical quivering movements, after 4 hrs of treatment, opercular movement increased, resting intermittently at the end of experiment.	Violent escapism, jumping resting, jerky movement on prodding, swimming upside down, at the end of 96 hrs.	Violent movements, fast gasping with increased opercular movement, loss of equilibrium, lying on lateral side at the end of the treatments.
Phosphamidon	Whirling movements, restless, upto initial 5 hrs, jerky movement on prodding, resting at the bottom of the tank.	same as in 0.01 ppm treatments.	Restless, whirling movement was high upto initial 6 hrs, followed by resting and jerky movement on prodding, lost balance, lying on the side with gulping.
Dichlorvos	Slow whirling movement, gasping, opercular movement increased slowly upto initial 48 hrs, then afterwards fishes came to normal.	Same as in the case of 0.01 ppm treatment upto 24 hrs, reduced activity with resting and jerking movement.	Same as in the case of 0.1 ppm upto 48 hrs, lying on lateral side and increased gasping, at the end of the 96 hrs equilibrium was lost.

Monocrotophos	Restless behaviour, irregular body and opercular movement upto 24 hrs then came to normal.	Violent movement, gasping, resting, lying on lateral side, jerky movement on prodding, swam slowly on their sides.	Vigorous escaping behaviour, whirling movement, gasping increased, loss of equilibrium at the end of 60th hrs of treatment, swam slowly on their side, resting on the bottom of the tank and gulping.
Malathion	Excitation, violent movement on prodding upto 5 hrs then fishes came to normal.	Same as in the case of 0.01 ppm treatment.	Violent swimming for initial 8 hrs, all fishes came to the surface at the end of 24 hrs, opercular movement increased, resting intermittently and swam slowly, lying on lateral side at the end of experiment.

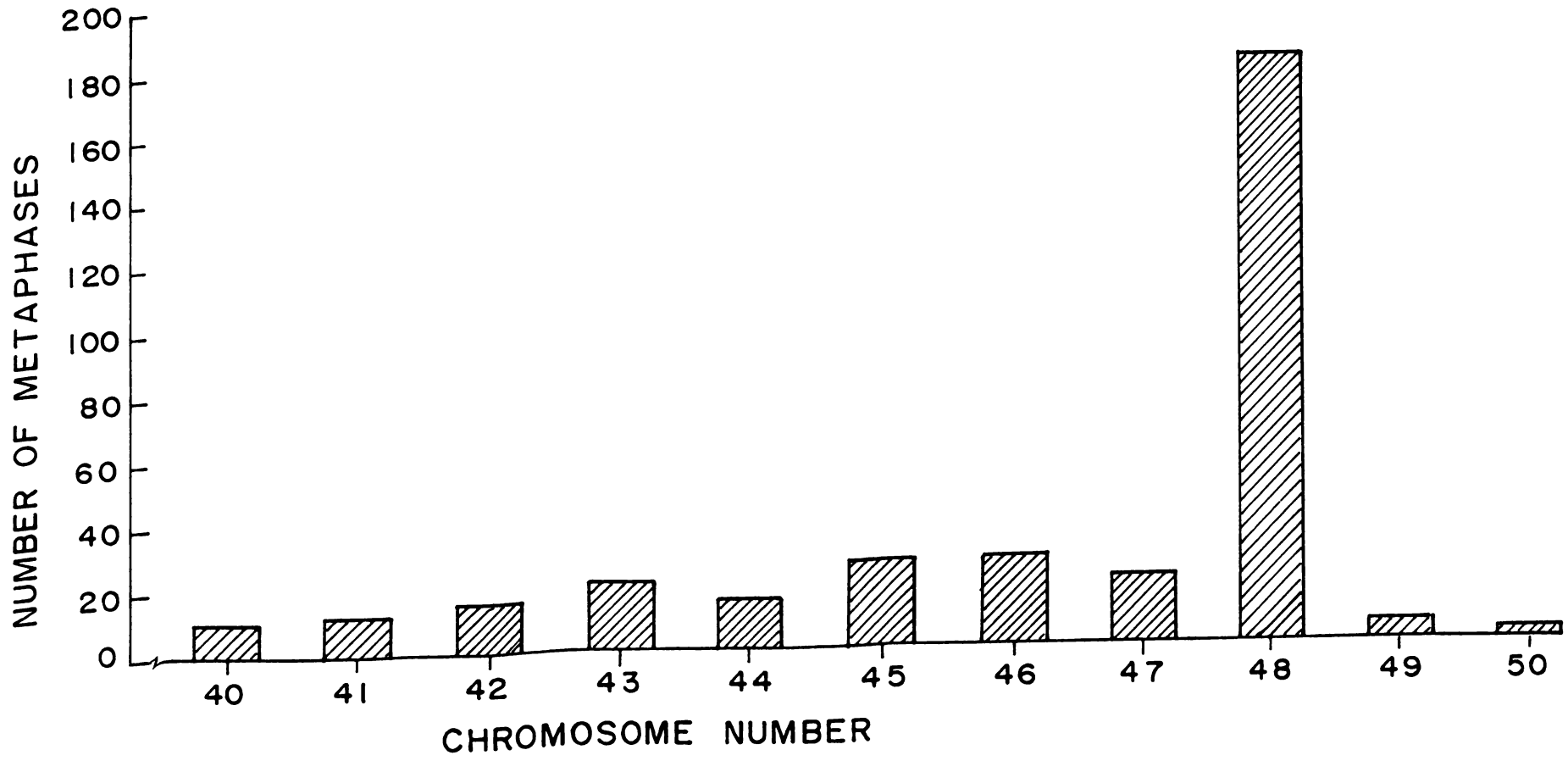

FIG. 1. FREQUENCY OF CHROMOSOME NUMBER OF *L. parsia*.

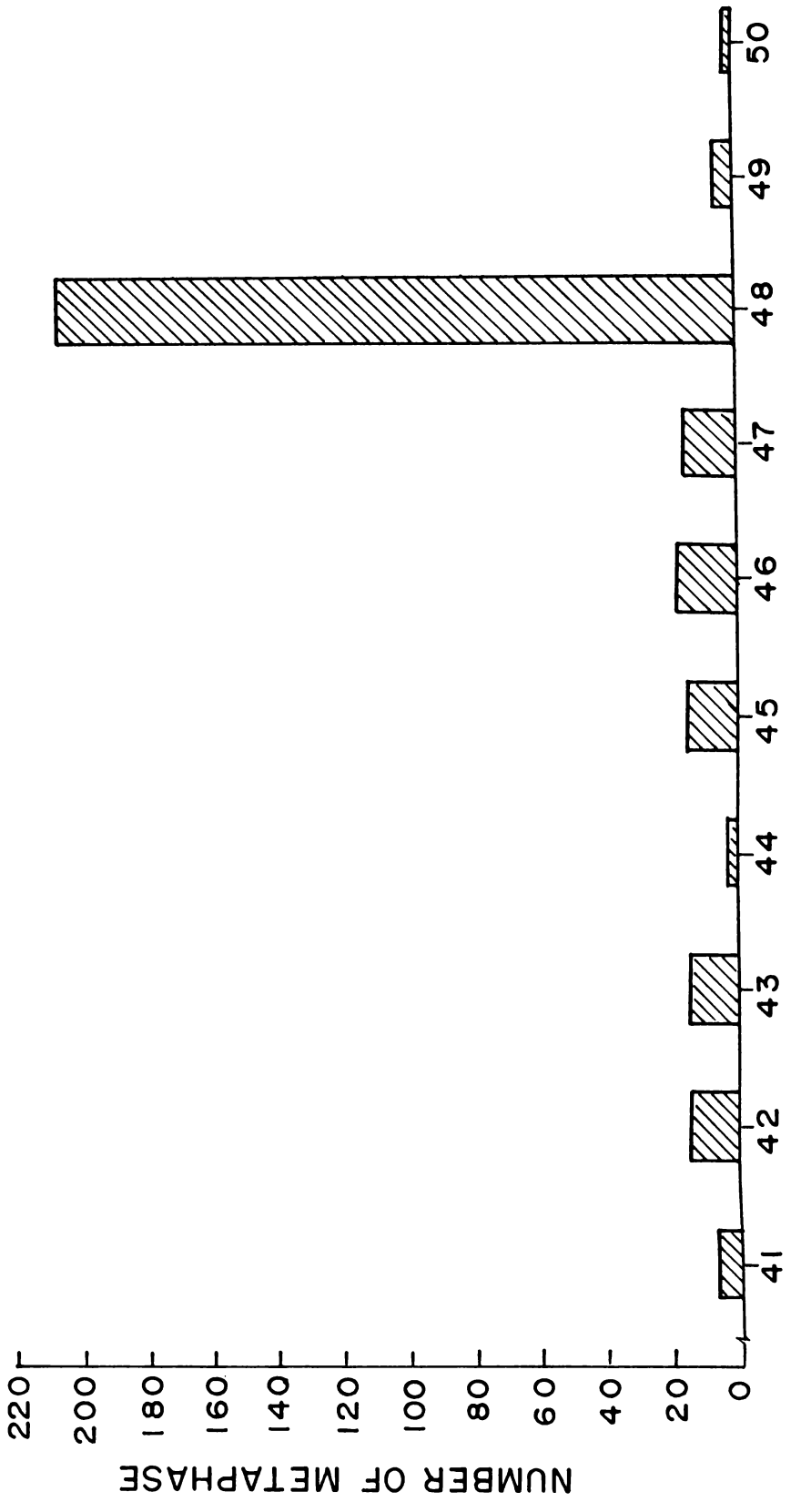

FIG. 2. FREQUENCY OF CHROMOS

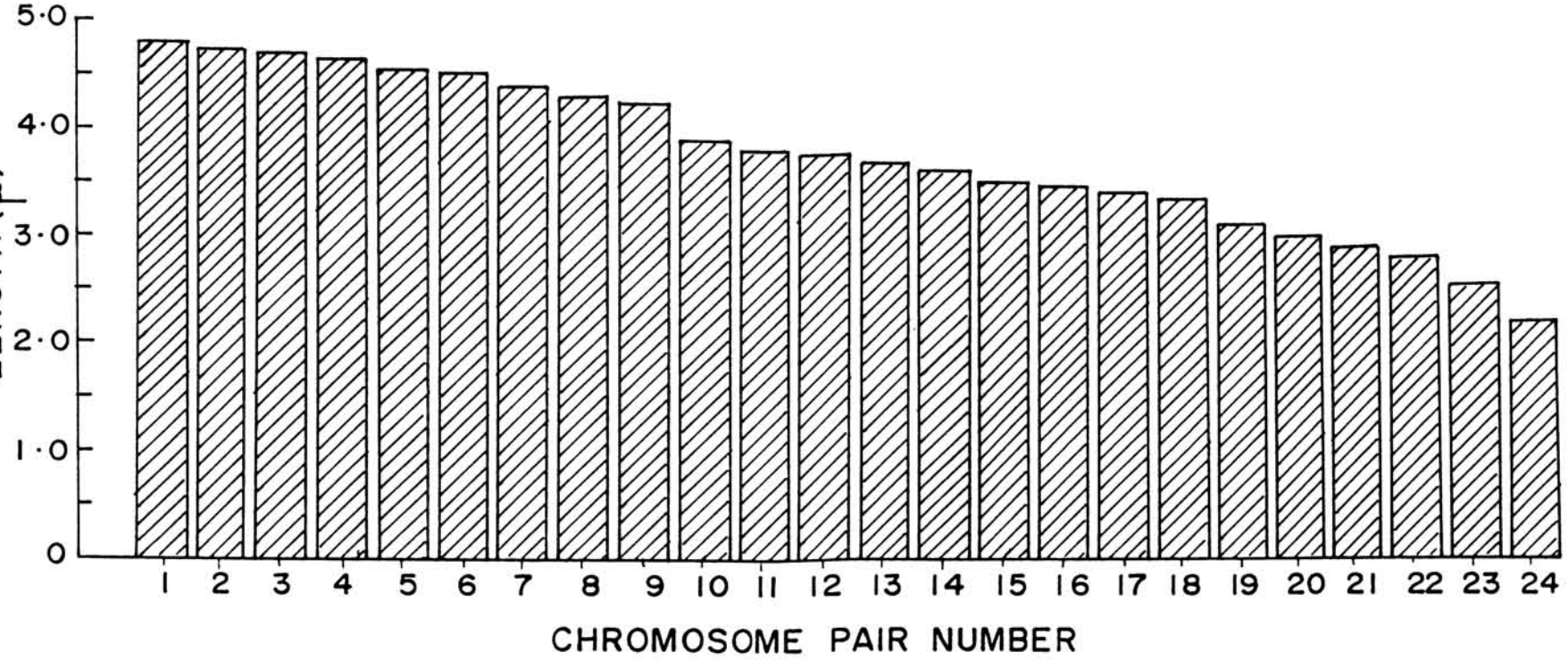

FIG.3. HISTOGRAM OF CHROMOSOME LENGTHS (μ) OF *L. parsia*.

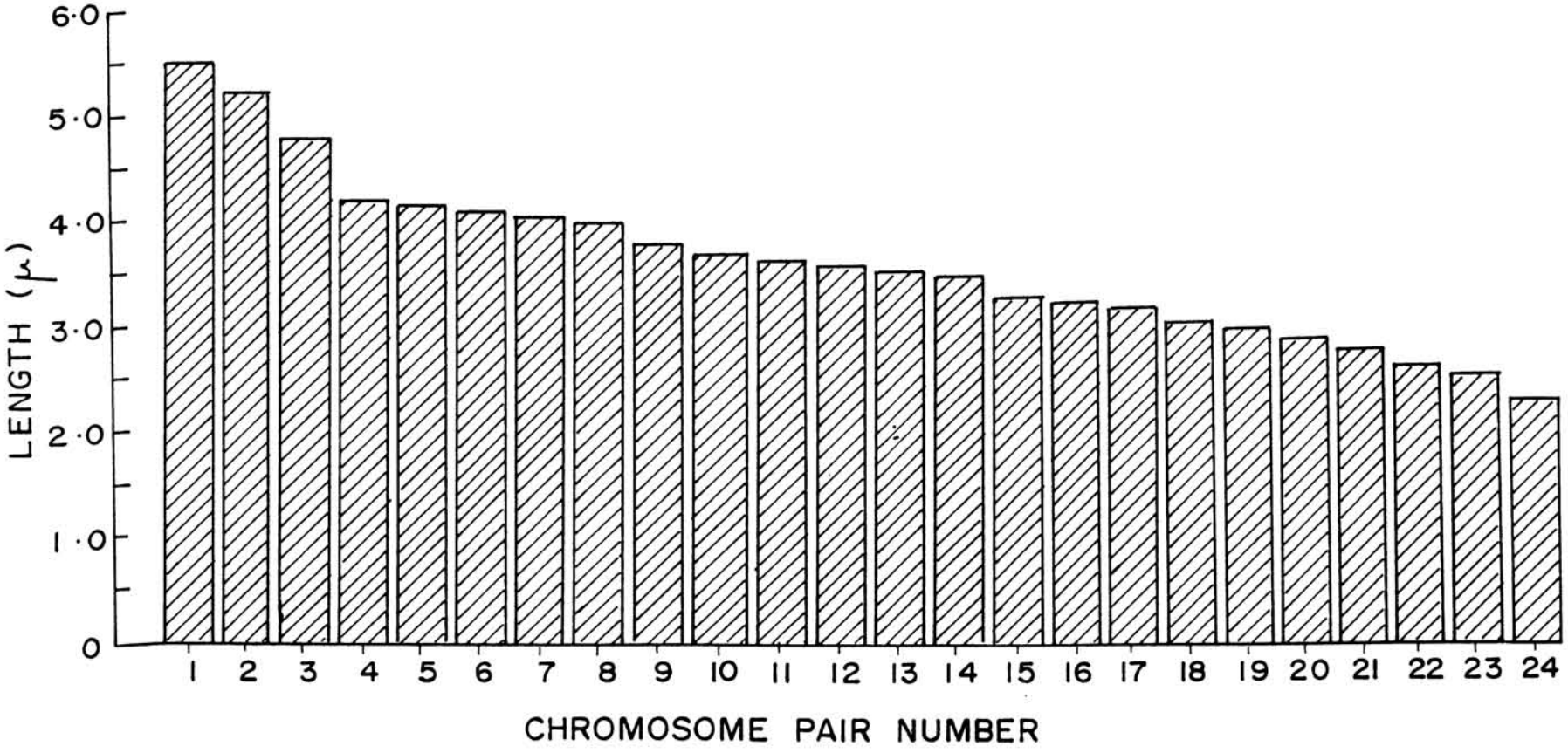

FIG. 4. HISTOGRAM OF CHROMOSOME LENGTHS (μ) OF *M. cephalus*

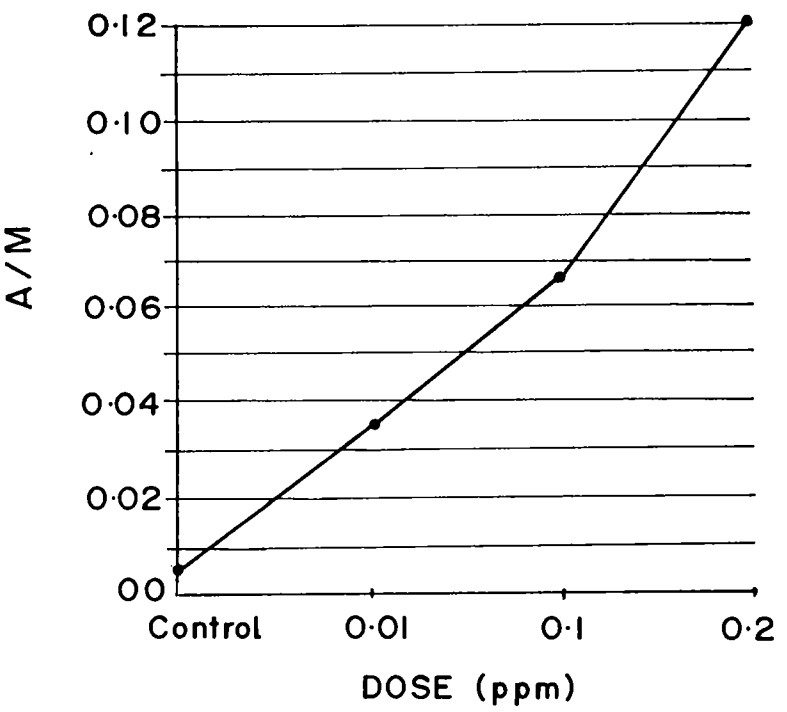

FIG. 5. ANALYSIS OF CHROMOSOME ABERRATIONS IN *L. parsia* EXPOSED TO METHYL PARATHION.
A/M – Aberration per metaphase

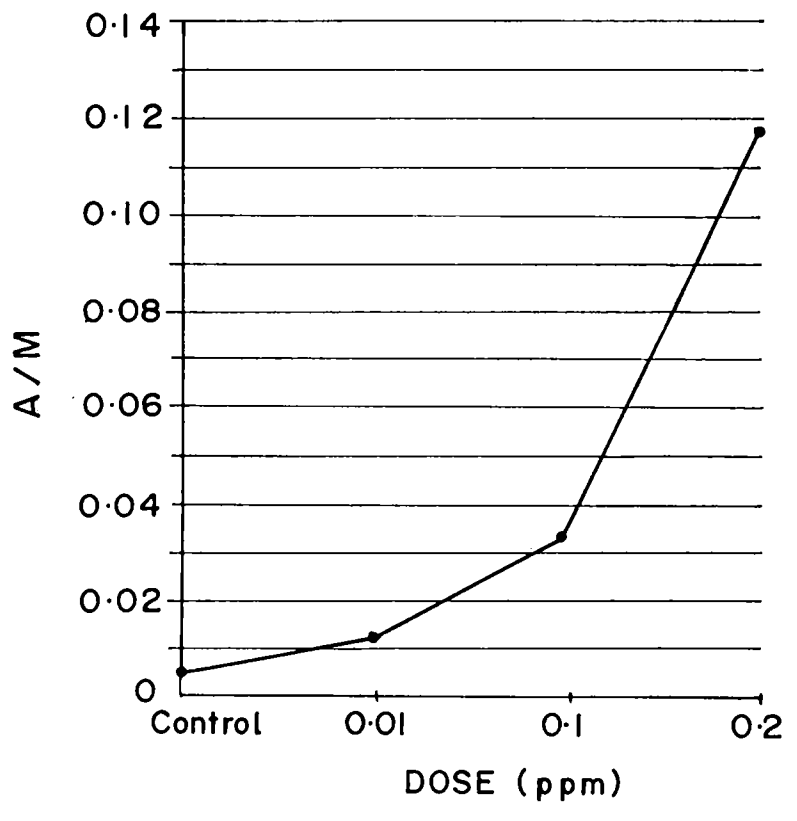

FIG. 6. ANALYSIS OF CHROMOSOME ABERRATIONS IN *L. parsia* EXPOSED TO PHOSPHAMIDON.
A/M – Aberration per metaphase

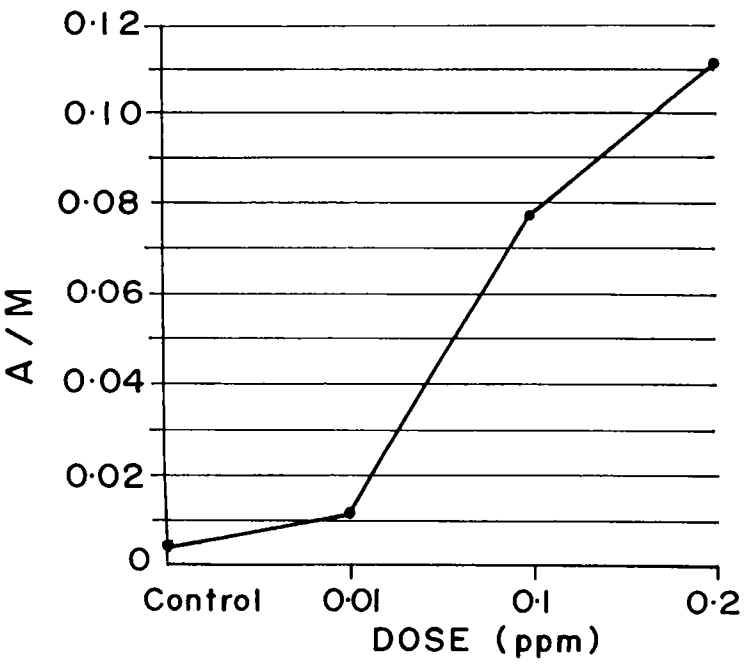

FIG. 7. ANALYSIS OF CHROMOSOME ABERRATIONS IN *L. parsia* EXPOSED TO DICHLORVOS.
A/M - Aberration per Metaphase

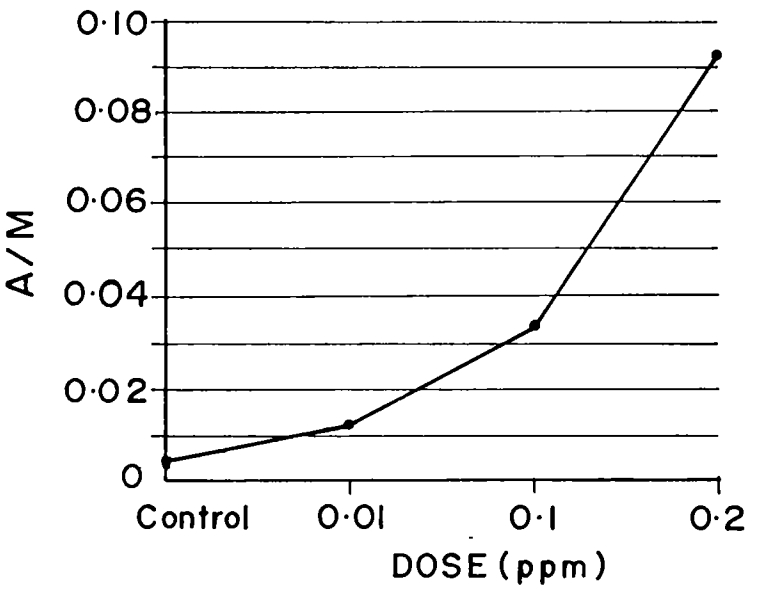

FIG. 8. ANALYSIS OF CHROMOSOME ABERRATIONS IN *L. parsia* EXPOSED TO MONOCROTOPHOS.
A / M – Aberration per Metaphase.

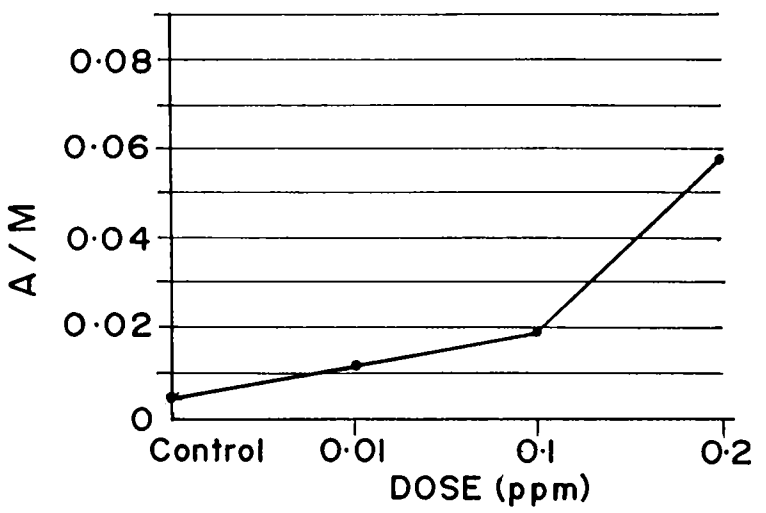

FIG. 9 . ANALYSIS OF CHROMOSOME ABERRATIONS IN *L. parsia* EXPOSED TO MALATHION.

A / M - Aberration per metaphase

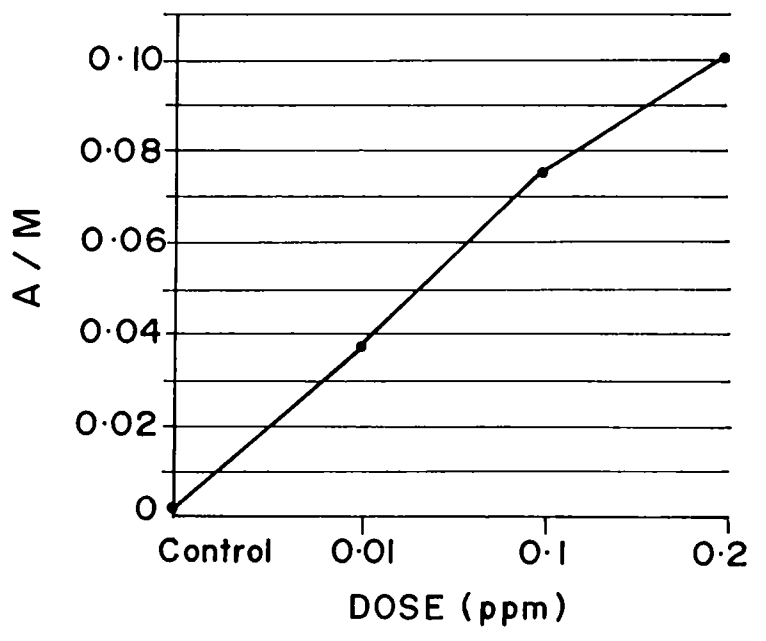

FIG. 10. ANALYSIS OF CHROMOSOME ABERRATIONS IN *M. cephalus* EXPOSED TO METHYL PARATHION.

A / M - Aberration per metaphase.

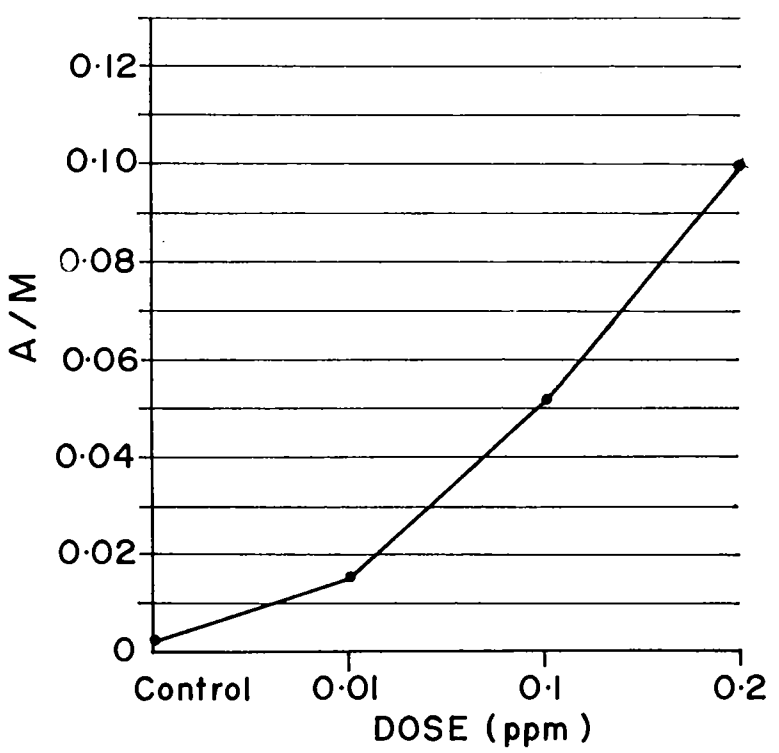

FIG. 11. ANALYSIS OF CHROMOSOME ABERRATIONS IN *M. cephalus* EXPOSED TO PHOSPHAMIDON.

A/M – Aberration per Metaphase

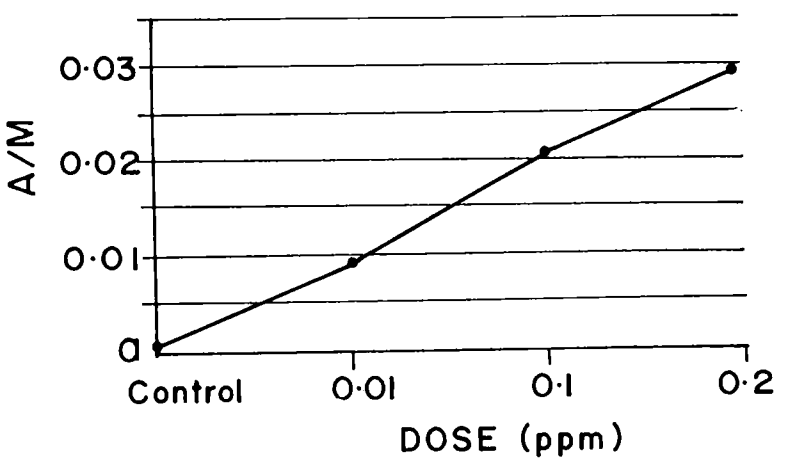

FIG. 12. ANALYSIS OF CHROMOSOME ABERRATIONS IN *M. cephalus* EXPOSED TO DICHLORVOS.

A / M – Aberration per Metaphase.

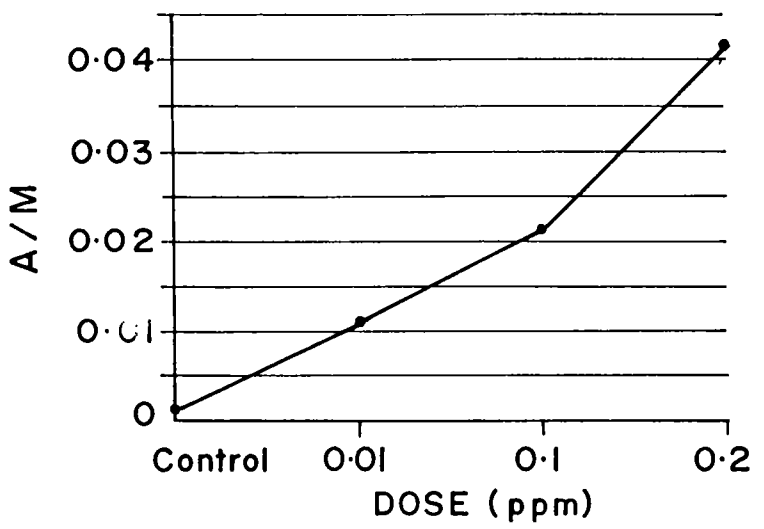

FIG. 13. ANALYSIS OF CHROMOSOME ABERRATIONS IN *M. cephalus* EXPOSED TO MONOCROTOPHOS.

A/M - Aberration per metaphase

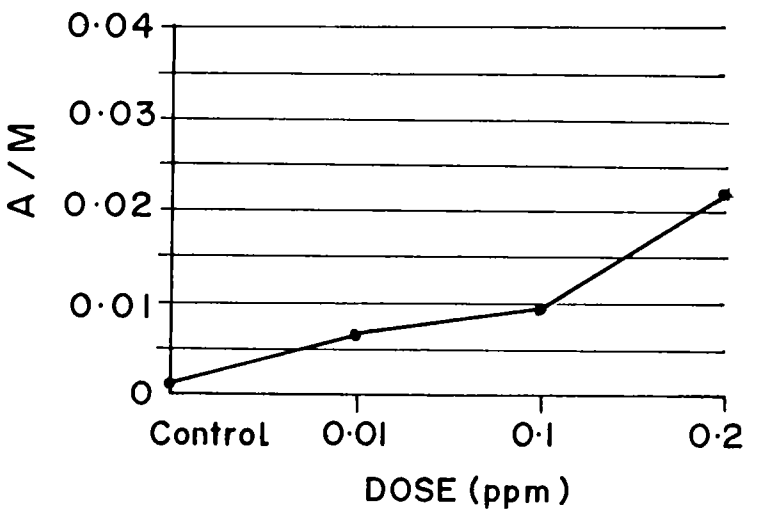

FIG. 14. ANALYSIS OF CHROMOSOME ABERRATIONS IN *M. cephalus* EXPOSED TO MALATHION.

A/M - Aberration per metaphase.

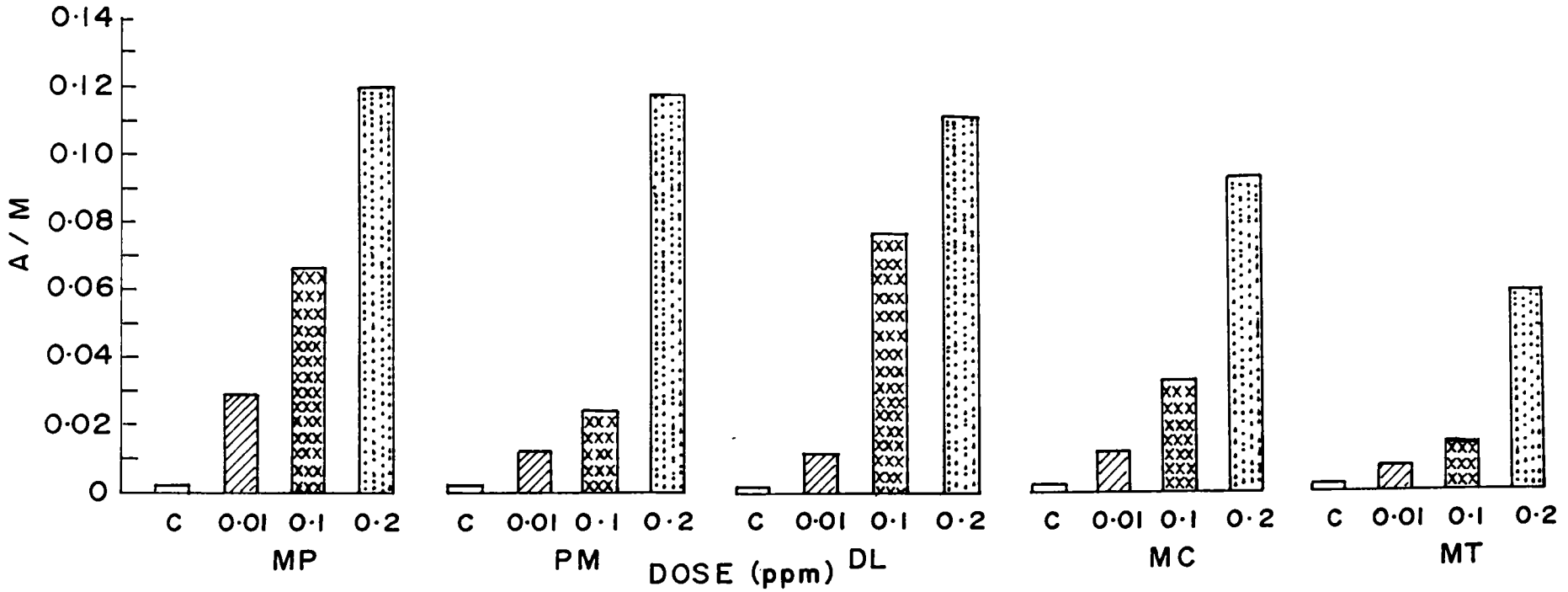

FIG. 15. COMPARISON OF INDUCED CHROMOSOME ABERRATIONS IN *L. parsia*.

C – Control MP – Methyl Parathion PM – Phosphamidon DL – Dichlorvos
MC – Monocrotophos MT – Malathion.

A / M – Aberration per Metaphase.

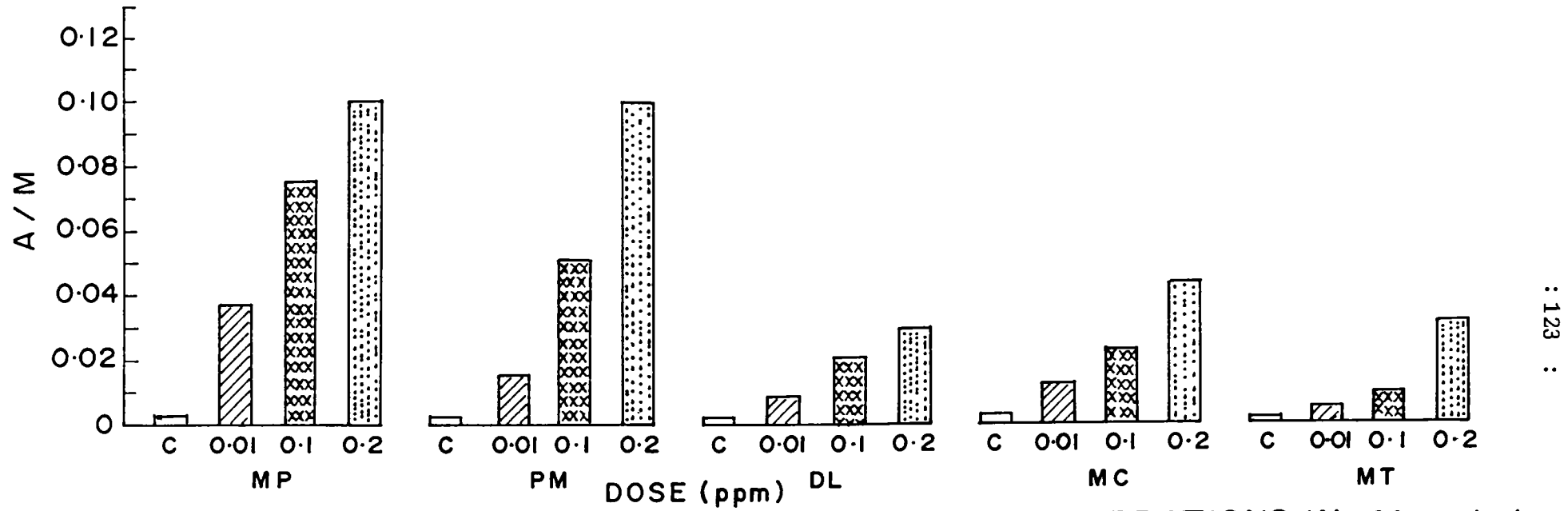

FIG.16. COMPARISON OF INDUCED CHROMOSOME ABERRATIONS IN *M. cephalus*.

C – Control MP – Methyl Parathion PM – Phosphamidon DL – Dichlorvos
MC – Monocrotophos MT – Malathion.

A / M – Aberration per Metaphase.

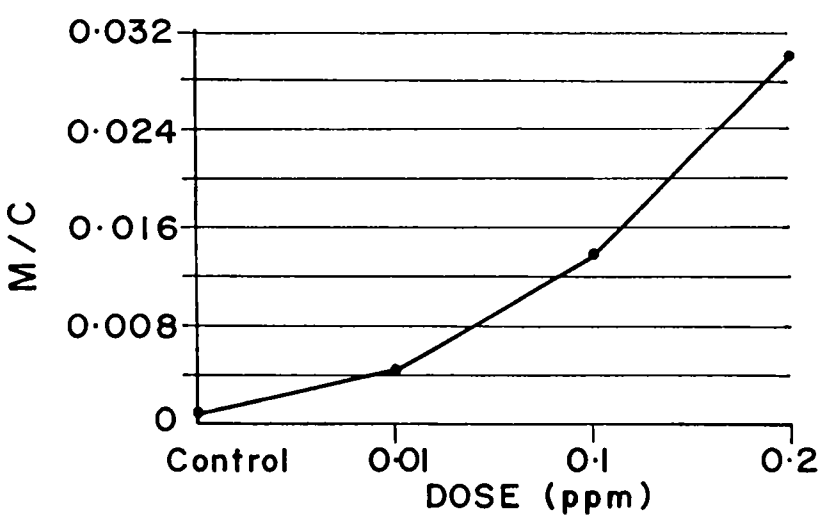

FIG.17. FREQUENCY OF MICRONUCLEUS IN *L. parsia* EXPOSED TO METHYL PARATHION.

M/C - Micronuclei per cell.

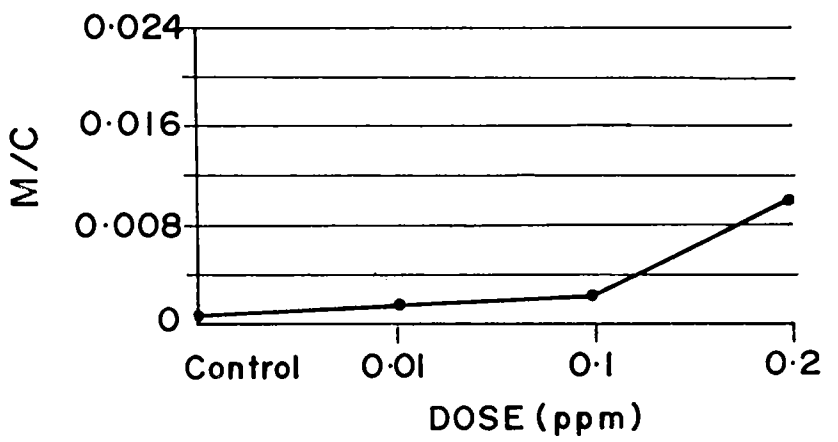

FIG.18 . FREQUENCY OF MICRONUCLEUS IN *L. parsia* EXPOSED TO PHOSPHAMIDON.

M/C - Micronuclei per cell.

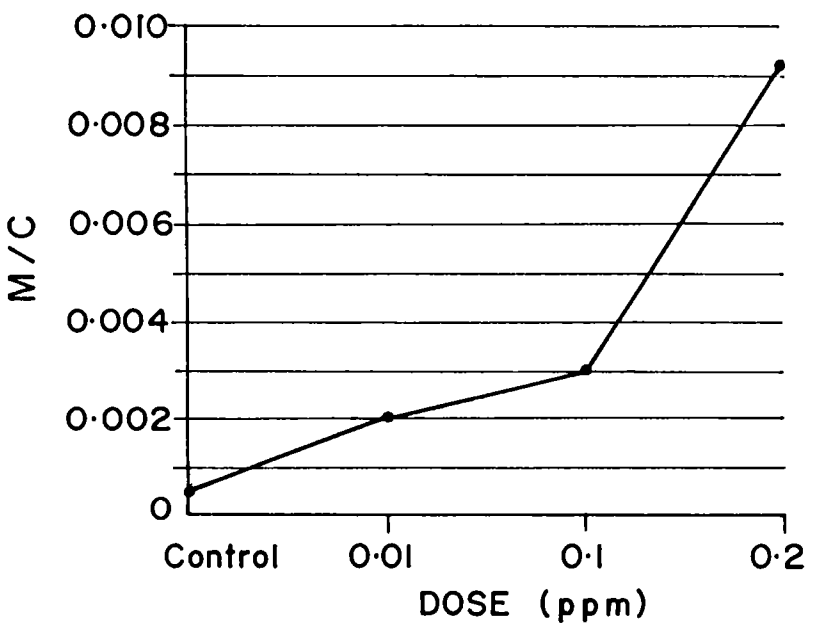

FIG.19. FREQUENCY OF MICRONUCLEUS IN *L.parsia* EXPOSED TO DICHLORVOS.
M/C – Micronuclei per cell.

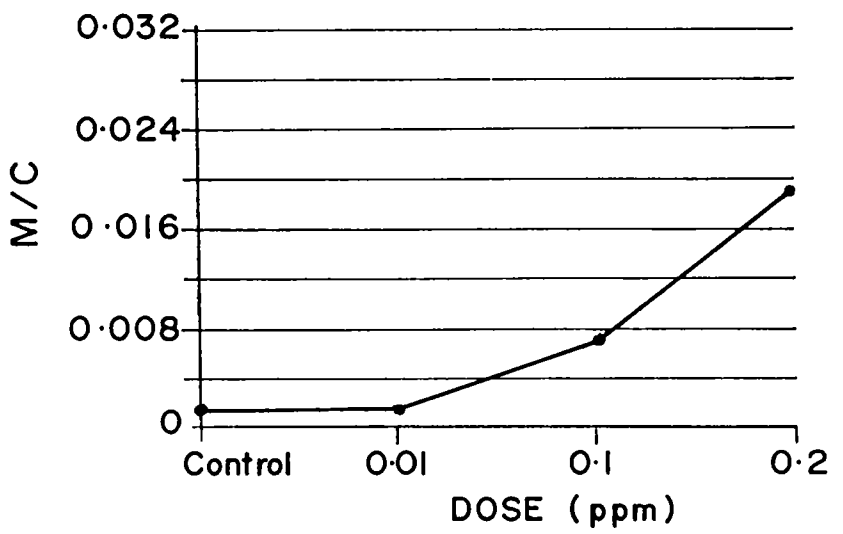

FIG.20. FREQUENCY OF MICRONUCLEUS IN *L. parsia* EXPOSED TO MONOCROTOPHOS.
M/C – Micronuclei per cell.

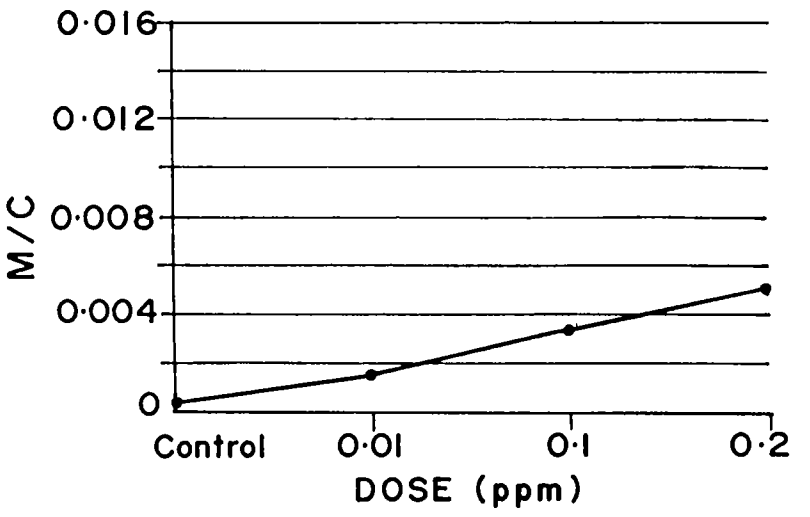

FIG. 21. FREQUENCY OF MICRONUCLEUS IN *L. parsia* EXPOSED TO MALATHION.

M/C - Micronuclei per cell.

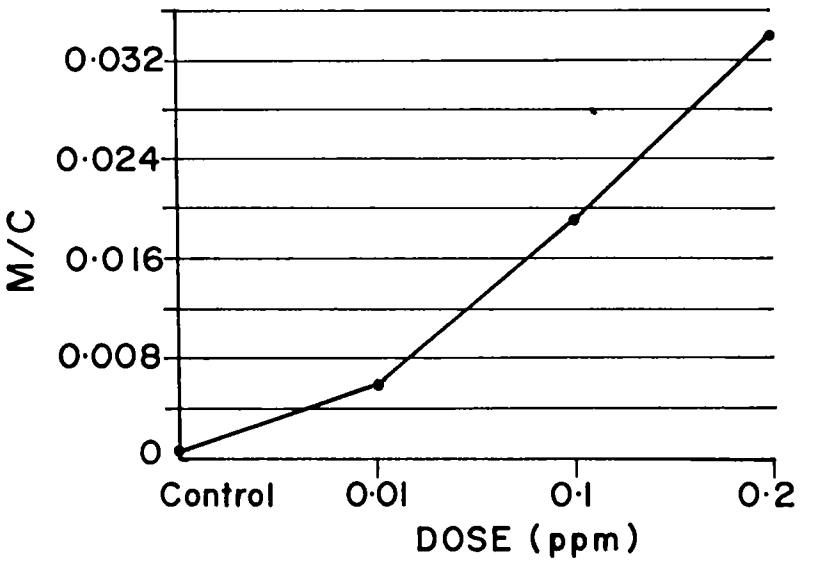

FIG. 22. FREQUENCY OF MICRONUCLEUS IN *M. cephalus* EXPOSED TO METHYL PARATHION.

M/C - Micronuclei per cell.

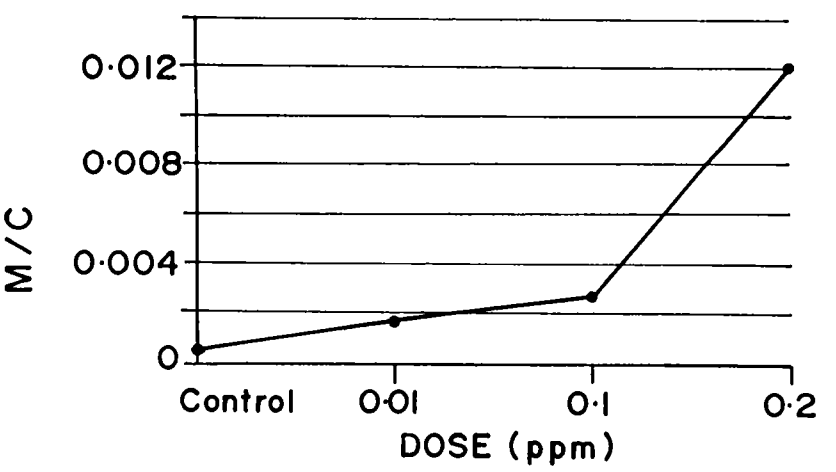

FIG. 23. FREQUENCY OF MICRONUCLEUS IN *M. cephalus* EXPOSED TO PHOSPHAMIDON.

M/C – Micronuclei per cell.

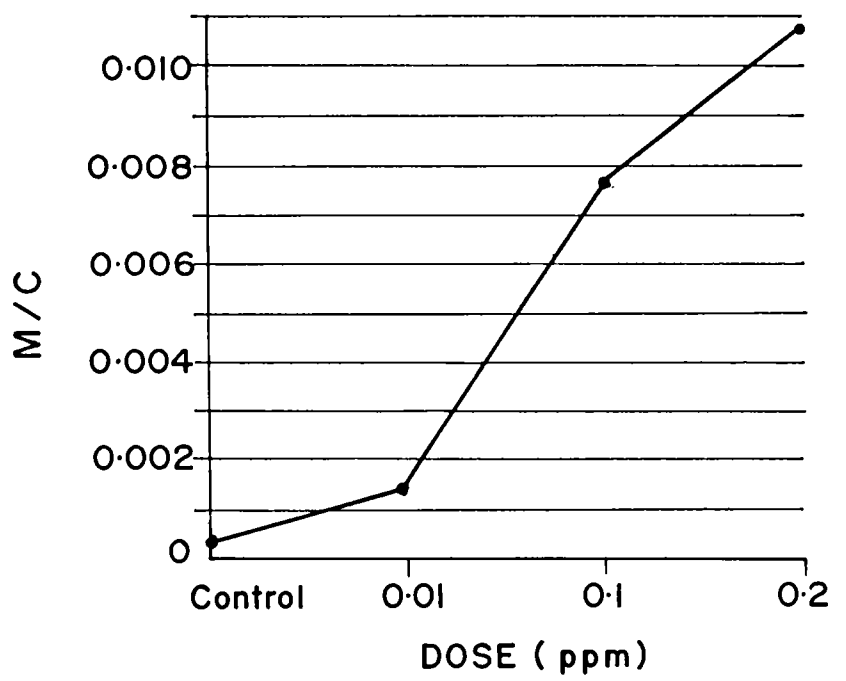

FIG. 24. FREQUENCY OF MICRONUCLEUS IN *M. cephalus* EXPOSED TO DICHLORVOS.

M/C – Micronuclei per cell.

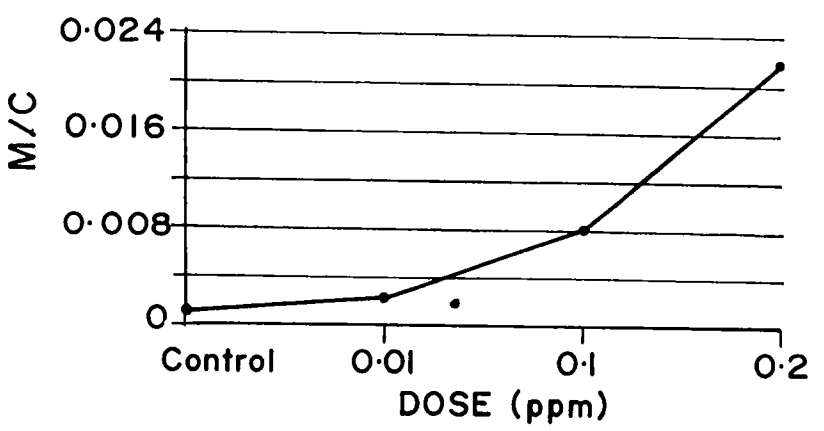

FIG. 25. FREQUENCY OF MICRONUCLEUS IN *M. cephalus* EXPOSED TO MONO-CROTOPHOS.

M/C — Micronuclei per cell.

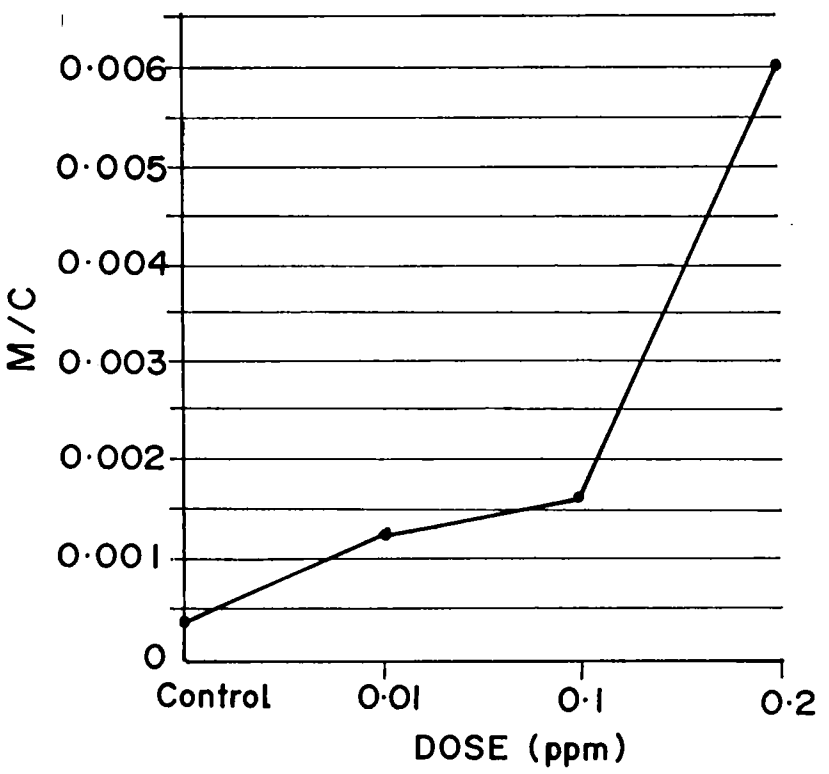

FIG. 26. FREQUENCY OF MICRONUCLEUS IN *M. cephalus* EXPOSED TO MALATHION.

M/C — Micronuclei per cell.

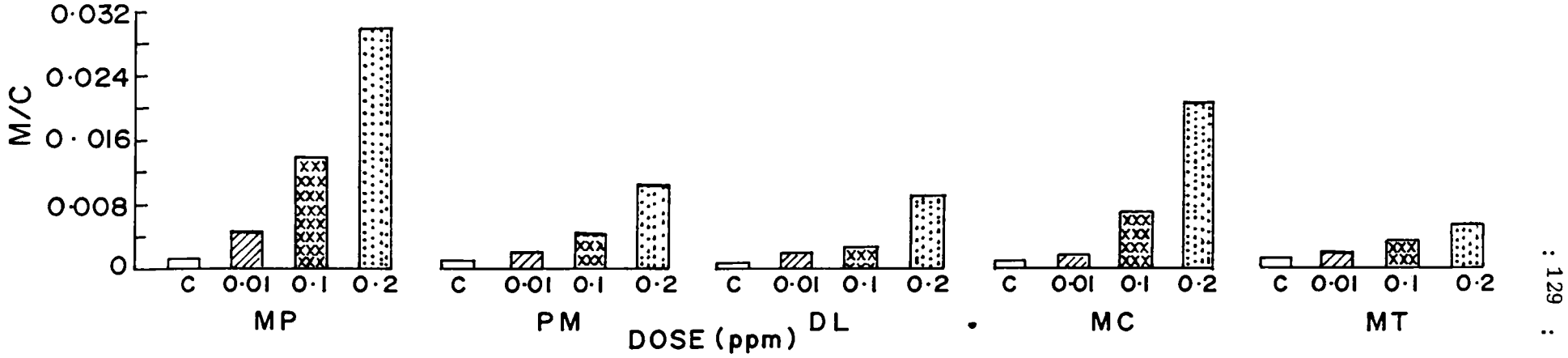

FIG. 27. COMPARISON OF FREQUENCY OF MICRONUCLEUS IN *L. parsia* EXPOSED TO VARIOUS PESTICIDES.

C – Control MP – Methyl Parathion PM – Phosphamidon DL – Dichlorvos
MC – Monocrotophos MT – Malathion.

M/C – Micronuclei per cell.

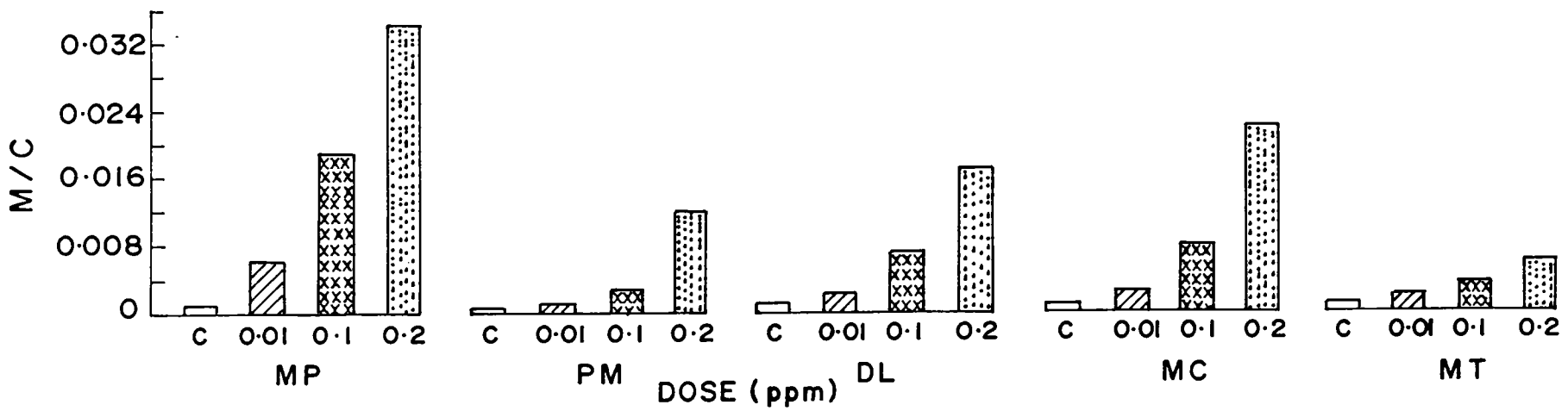

FIG.28. COMPARISON OF FREQUENCY OF MICRONUCLEUS IN *M. cephalus*. EXPOSED TO VARIOUS PESTICIDES.

C – Control MP – Methyl Parathion PM – Phosphomidon DL – Dichlorvos
MC – Monocrotophos MT – Malathion.
M / C – Micronuclei per cell.

D I S C U S S I O N

1. SELECTION OF CANDIDATE SPECIES

Fishes have been successfully used to demonstrate the presence of genotoxic agents in aquatic environment (Prein et al., 1978, Alink et al., 1980, Hooftman and Vink, 1981). Various species of fishes such as Zebra fish, Brachydanio rerio (Endo and Ingalls, 1968), Black ghost Knifefish, Apteronotus albifrons (Howell, 1972), Umbra limi, U.Pygmae and N.rachowi (Kligerman et al., 1975, Kligerman, 1979, Prein et al.,1978, Alink et al.,1980 Kligerman et al., 1984, Kerkhoff and Gaag, 1985), Guppy, Poecilia reticulata (Mathew et al., 1978), Boleophthalmus dussumieri (Krishnaja and Rege, 1982) and gonad cells of rainbow trout and fry tissue of bluegill (Kocan et al., 1985b), and Polychaeta N.arenaceodentata (Pesch and Pesch, 1980) have already been used as model for genotoxicity study. But, Pankaj et al., (1990) suggested that common carp, Cyprinus carpio could be considered as less suited for chromosome aberration study because it has large diploid number (2n = 98 ± 4) and small size chromosomes.

For the present study, Lates calcarifer, Scatophagus argus. Liza parsia and Mugil cephalus were selected to screen for model species. Of these, L.parsia and M.cephalus were found to be suitable species, because these two species have small diploid number (2n=48), all chromosomes are acrocentric and more or less of same size, commercially important, easily acclimatised in laboratory conditions,

and available in polluted and non polluted waters. But, in the case of L.calcarifer, even though the diploid number of chromosome (2n = 48) was small, the chromosome configuration was 2 - metacentric, 6 - submetacentric, 2 - subtelocentric and 38 acrocentric, and sufficient number of fish was not available in polluted area. Hence, this species was not selected for the present study. Though the diploid chromosome number (2n = 48) in S.argus was small and all chromosomes were acrocentric, it was not selected for the present study because it was not occuring in sufficient numbers in polluted area.

2. CHROMOSOME PREPARATION

Various methods have been suggested by different workers (Ford and Hamerton, 1956; Mc phail and Jones, 1966; Chen and Ebeling, 1968; Stewart and Levin, 1968; Denton and Howell, 1969; Le Grande and Fitzsimons, 1976; Kligerman and Bloom, 1977; Chourrout and Happe, 1986; Reddy and John, 1986; etc.). Although a number of methods have been evolved for chromosome preparation in fishes, standardisation had to be conducted for obtaining well spread chromosome with clear morphology. In most of the methods, the cells were deposited on the slides and dried by air drying or flame drying. For the present study, different methods were employed to achieve a suitable methodology for the candidate species, L.parsia and M.cephalus. The steps of chromosome preparation such as colchicine treatment, hypotonisation, fixation, cell suspension,

deposition of cells on slides, etc., were modified to obtain the best results. Air drying, flame drying and squash methods were tried, out of which, air drying was found to be the suitable method to prepare the chromosomes in these fishes. The methods adopted by Chen and Ebeling (1968), Denton and Howell (1969), Kligerman and Bloom (1977) and Reddy and John (1986) were found to be suitable for chromosome preparation. No good metaphase was obtained in Mc phail and Jones (1966) method. This may be due to manual squashing.

More numbers of metaphase spreads were obtained when intramuscular injection of colchicine were administered at 0.01% concentration for 2½ hrs exposures @ 1 ml/100 gm of body weight of fish. Khuda-Bukhsh and Manna (1976 a) injected 0.1% colchicine solution intramuscularly at the rate of 2 ml/100 g body weight for 4 hr exposure to prepare the chromosome in Mugil corsula and M.parsia. Shortening of the chromosome was observed when higher doses of colchicine were given. Metaphase plate number per individual was found to be less when the colchicine exposure time was less. This may be due to incomplete action of colchicine. Number of metaphase was found to be higher when the fishes swimmed very actively after the colchicine injection.

Potassium chloride and Sodium citrate solution in the concentration of 0.4%, 0.8%, 1%, 2%, 5% were separately tried for hypotonic treatments for 15, 30, 45 and 60 minutes duration. Of these,

1% sodium citrate solution for 30 minute treatment gave optimum hypotonisation. Quick hypotonic action was obtained under cold conditions. Bantock and Cockayne (1975) reported that methanol used in fixation has helped to improve the chromosome morphology. However, the present study does not support this observation. But, acetic acid in cell suspension improved the chromosome morphology.

Fixation in Methanol:acetic acid for 2 hours was found to be sufficient. Prolonged storage of fixed tissue in cold condition (refrigerator) did not create any problem to obtain metaphase. In the present study, overnight fixed tissues were also used. Before preparing the cell suspension, the tissue was changed into fresh fixative.

Maximum stained chromosomes were obtained at 4% Giemsa solution in 0.1 M phosphate buffer (pH 6.8) for 18 to 20 minutes. High concentration of Giemsa and prolonged treatment did not improve the staining.

Since DPX mounted slide showed fading in normal chromosome after a few days, slides were prepared and observed without mounting. All slides were screened immediately after preparation.

3. DIPLOID NUMBERS

L.parsia: The diploid number of 48 chromosome in M.parsia collected from West Bengal was reported by Khuda Bukhsh and Manna (1976 a). In L.parsia collected from Cochin (west coast of India) for the present study also had diploid number 48. The diploid number of 48 in L ramada, L.aurata L.saliens were also reported by Cataudella, and Capanna(1973) and Cataudella et al.(1974).

M.cephalus: The diploid number of 48 chromosomes in M.cephalus is confirmed with earlier studies of Cataudella and Capanna (1973), Chatterjee and Majhi (1973), Cataudella et al. (1974) , Le Grande and Fitzsimons (1976). The diploid number of 48 in Mediterranean species, Mugil cephalus, Chelon labours, Liza ramada, L.saliens, L.aurata and Oedalechilus lebeo were reported by Cataudella and Capanna (1973) and Catudella et al. (1974), whereas the diploid number of 24 in Mugil corsula has been reported by Nayyar (1966).

4. . ANALYSIS OF KARYOTYPES

L.parsia: In the present study all chromosomes are acrocentric as reported in previous study. There is not much variation among the chromosome (Plate 4 and Fig.3). The length of chromosomes varied from 4.800 to 2.125 μ and relative length is 5.332 to 2.361% (Table 5). But Khuda Bukhsh and Manna (1976 a) reported that mean length of longest and shortest pairs of chromosome of M.parsia was

between 1.68 μ and 0.80 μ. No heteromorphic sex chromosomes were reported in the candidate species.

M.cephalus: Karyotype of this species consisted of 48 acrocentric chromosomes. There is not much variation among these chromosomes (Plate 5 and Fig.4). No heteromorphic sex chromosomes were observed. Longest chromosomes of this species is 5.5280 μ and smallest chromosomes 2.2505 μ and relative length varied from 6.402 to 2.606%.

Le Grande and Fitzsimons (1976) reported that diploid number of M.curema was 28 and chromosome configuration was 10 pairs of metacentric, 2 pairs of subtelocentric and 2 pairs of acrocentric. This species was distinctively different from other mullets. In the present study, such type variation was not found in both test species. Nayyar (1966) suggested that the primitive teleost karyotype consisted of 46-48 chromosomes. According to his report the karyotype of test animals in the present study are considered to be of primitive teleost.

Fishes exhibit chromosome variation from population to population (Junxiu, 1983). The chromosome complements of Galaxias maculatus from New Zealand differs from that of Australian and Chilean specimens (Merrilees, 1975). According to his reports of karyotype of this species showed distinct variation of karyotype of Chilean specimens. Such type variations are not found in test animals especially Mediterranean and Indian species which showed same diploid

number and type of chromosome. Intra individual chromosomal polymorphism was observed in rainbow trout by Ohno et al. (1965) and Thorgaard (1976); in Mystus vittatus by Manna and Prasad (1976).

Homologous chromosome is genetically different from all other chromosome pairs in the same nucleus. This may cause the morphological phenotype difference including differences between chromosome pairs in relative size, shape and centromere position. The diploid (2n) karyotype of 48 acrocentric chromosome is found throughout several diverse orders of sub class Teleostei (class Osteicthyes) and seemed to be predominant karyotype in Perciformes (Robert, 1964, 1967; Denton, 1973; Chairelli and Capanna, 1973).

5. OBSERVATION OF MICRONUCLEI

Jones and Parry (1992) reported that the micronuclei are small membrane bound mass of chromatin which may contain acentric fragments of chromosome materials or centric fragments or whole chromosome.

Longwell et al., (1983); Das and Nanda (1986); Al-Sabti (1986 a); Hose et al., (1987) and Long and Buchman (1989) indicated that their data supported the use of piscine micronucleus test as a biological indicator of exposure to pollutants. The present study also agreed with their suggestion. Various methods like, Schmid (1976); Salamone et al. (1980); Hooftman and de Raat (1982); Walton et al. (1984); Manna and Sadhukhan (1986) and Hose et al. (1987) were tried to get good spread of cells and clarity between the cytoplasm and nucleus, Smit and Hattingh (1980) used sodium citrate as anticoagulent. But, in the present study either sodium citrate or haparin was not used, because it badly affected the cells by hypotonic action which lead to more artifacts. To avoid the clotting soon after neat amputation in caudal region, blood was immediately smeared on clean slides and were allowed to air dry before fixation in absolute methanol and stored in dark container until the completion of sampling as done by earlier workers (Schemid, 1976 and Carrasco et al., 1990). The smeared slides were stained in May-Grunwald and followed by 2% Giemsa in 0.1 M Sorenson's buffer (pH 6.8) for 20 minutes as described by Hose et al. (1987). Good contrast between cytoplasm and nucleus of erythrocytes were obtained, when Schmid (1976), Walton et al. (1984) and Hose et al. (1987) methods were followed, Hooftman and de Raat (1982) reported the Fuelgen's methods which did not help to get the satisfactory results in the present study. Das and Nanda (1986) and Manjone et al. (1988)

reported lower frequency of micronuclei in Fuelgen's methods than Giemsa method. Overend and Stacey (1947) reported that Fuelgen stain caused the hydrolysis of genome material which yielded free aldehyde group at DNA level and its affect also lead to RNA. Therefore, there was some possibility to distroy the DNA fragments in micronuclei. This may be the reason to get the micronuclei at adequate level in the present study.

Manna and Sadhukhan (1986) reported wright stain method which did not give satisfactory results. May-Grunwald staining followed by Giemsa staining gave best results in the present study. Since DPX mounting medium, enhanced the fading of nucleus, the stained slides were observed without mounting.

Chromosome aberration measurements were shown to be a better parameters than Sister Chromatid Exchange (SCE) in environmental studies (Thilager and Kumargo, 1983). Hence present study is mainly on the chromosome aberration in L.parsia and M.cephalus.

6. BASE LINE VALUES

Chromosome aberrations: The base line chromosome aberration was shown by almost all species. Base line chromosome aberrations were reported from different species by many workers. Kilgerman et al. (1975) reported 0.03% base line chromosome aberration in the

kidney tissue of U.limi. Mong and Berra (1976) also reported 1.5% base line chromosome aberrations in gill tissue of U.limi whereas Hooftman (1981) reported zero aberration in gill tissue of N.rachowi in one set of experiments. But, in another set of experiments, gill tissue of this species showed 0.5% base line aberrations. Krishnaja and Rege (1982) observed the 0.0017 and 0.0033 of aberration per metaphase in gill tissue of B.dussumieri in two sets of experiments. The base line chromosome aberration per cell authentically reported in kidney tissues of C.idella was 7.3; 6.4 in T.tinca and 5.8 in C.carpio as reported by Al-Sabti (1985a, 1986b). In in vitro study, 8-13% of base line aberrations in gonad tissue of S.gairdneri were reported by Kocan et al.(1985b).

The base line chromosome aberrations per metaphase in the present study were found to be 0.0055 in L.parsia and 0.0042 in M.cephalus (Table 7&8). The base line chromosome aberrations were confirmed when 1083 metaphases were examined in L.parsia whereas 950 metaphases were examined in M.cephalus. The present study emphasised that low base line aberrations were shown by the two candidate species (L.parsia and M.cephalus) in the form of gaps.

Micronuclei: Hooftman and de Raat (1982) reported that the base line micronuclei value in U.pygmaea was 0.07% in one set of experiments. But, in another set of experiments zero value was obtained. Heddle et al. (1983) observed the micronuclei frequencies of fishes averaging from 0.06-0.8%. But, Manna and Sudhukhan (1986) reported 0.1% of base line micronuclei in O.mossambica. Hose et al. (1987) reported the micronuclei frequency of 0.8% in fishes from control site. Van Hummelen et al. (1989) reported 1 micronucleus/ 1000 erythrocytes in larvae of Xenopus laevis. Rahman and Khuda Bukhsh (1992) reported 0.05% micronuclei as base line values in O.mossambica in one set of experiments and in other two sets of experiments it was 0.025% and 0.02%. But, micronuclei formation were not observed in C.punctatus. The present study is also shown that 0.0006 micronuclei per cell (0.06%) of L.parsia as base line value (Table 19) In M.cephalus, the baseline value of micronuclei was 0.0004 per cell (0.04%) (Table 20). Five thousand cells of each individual were examined in both candidate species.

TREATMENTS

Pesticides form an important group of environmental pollutants, widely used in agriculture, many of which are reported to be mutagenic (Durhma and Williams, 1972; Sandhu et al., 1985 and Waters et al., 1982). Pesticide factories are situated on the banks of the Cochin backwater and the effluents are discharged into this estuarine system. More over, the pesticides which were used in the present study, are commonly used in the agriculture sector in Kerala, which may be leached from paddy field to Cochin estuarine system. Anderson (1960) and Loosanoff (1960) also reported that pollution in aquatic system has been increased many a fold and these were found to be harmful to the fishes and other aquatic organisms. Among the pesticides, organophosphorous are widely used due to its superior effects (Galli et al., 1994) and the breakdown product of organophosphorous are more persistent than its parent compound (Somasundaram et al., 1987). Considering the above mentioned effects of pesticides on fishes, organophosphorous pesticides such as methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion which are widely used in the agriculture sector in Kerala, have been selected for the present study to investigate their effects on behavioural changes and the chromosomes of L. parsia and M. cephalus

a) Methyl Parathion 50% EC (Metacid-50): The effects of methyl parathion on the gill tissue of L.parsia and M.cephalus after exposure for 96 hrs are shown in Table 9 and Fig.5 and Table 14 and Fig.10. The identical doses (0.2, 0.1 and 0.01 ppm) significantly induced the chromosome aberration in L.parsia and M.cephalus which was observed after 250, 225, 202 metaphase examined as against the 361 metaphase

of control fishes (L.parsia) and 271, 252, 212 metaphase examined as against 475 metaphase of control fishes (M.cephalus). The aberration per metaphase were 0.0346, 0.0666 and 0.1200 respectively as against control value of 0.0055 per metaphase of L.parsia and 0.0377, 0.0753 and 0.1033 respectively as against the control value of 0.0042 per metaphase of M.cephalus. The aberration frequencies were found to be increased from lower dose to higher dose in two test animals. The effect is more or less same in these two species. Gaps, breaks, fragments, rings, isochromatid gaps and breaks, chromosome fragments were commonly observed in both the species. Aberrations in chromosome were observed near the centromere. The same location of aberration was observed in chromosome of B.dussumieri after exposure to mitomycin C by Krishnaja and Rege (1982).

Van Hummelan et al. (1989) used xenopus larvae to detect the induction of micronuclei after exposure to the alkylating agents ethylmethyl sulfonate and polycylic aromatic hydrocarbon benzo (a) pyrene. Rahman and Khuda Bukhsh (1992) reported that industrial effluents and chemical pollutants induced the formation of micronuclei in O. mossambica and C. punctatus. The present study also agreed with these reports.

The response of L.parsia and M.cephalus in micronuclei formation after treatment of methyl parathion showed increase from lower dose to higher dose (Table 21 & 26 and Fig.17 & 22). In all doses of this pesticide, the MN formation was statistically significant as

compared to the control. In individual, 3,000 cells were observed to study the micronuclei formation as against the control fishes of 5,000 cell in L.parsia and M.cephalus The number of micronuclei per cell was 0.0046 in lowest dose, 0.0140 in middle dose and 0.0303 in highest dose respectively as against the control value of 0.0006 per cell in L.parsia and 0.0060 in lowest dose, 0.0196 in middle dose and 0.0346 in highest dose as against the control value of 0.0004 per cell in M.cephalus. The micronucleus was attached near the main nucleus in almost all cases.

L.parsia and M.cephalus showed various physical reactions when exposed to different concentrations, 0.2 ppm (highest),0.1 ppm (middle) 0.01 ppm (lowest) of this pesticide (Table 33 & 34). The response of the fishes in the highest concentration was comparatively more than the middle and lowest concentration. Thus the present study implies that the intensity of physical response of fishes depends upon the concentration of pesticides. Welsh and Hanselka (1972) reported that methyl parathion induced the behavioural changes in the siamese fighting fish B.splendens whereas its effects on the locomotor orientation of gold fish C.auratus were reported by Rand et al. (1975). The present study also agreed with these reports. Farr (1977) reported the impairment of antipredatory behaviour in P.pugio when exposed to methyl parathion due to its anticholinesterase activity. In the present study also the fishes exhibited same physical reaction and this

behavioural changes of tested fishes may be due to the blocking of cholinesterase enzyme by methyl parathion, which are in part essential for normal neural transmission. The similar reasoning was also reported by Wild (1975). So there is some possibility to impair the antipredatory behaviour of L.parisa and M.cephalus due to the effect of this pesticide.

b) Phosphamidon 85% SL (Dimecron):

The response of L.parsia and M.cephalus to Phosphamidon after 96 hr exposure showed in Table 10 & Fig.6 and Table 15 & Fig.11. Selenium induced large number of breaks followed by mercury and chromium in the direct exposure on Boleophthalmus dussumieri (Krishnaja and Rege, 1982). The same type aberrations like gaps, breaks, fragments were commonly observed in the present study. Diamond and Clark (1970), Bourne and Jones (1973) reported that cells from different species responded differently to the same chemicals. The present study also agreed with this observation. The centromeric separation was observed only in the case of M.cephalus while isochromatid gaps were observed in L.parsia. The aberration per metaphase were 0.0129 in lowest dose, 0.0330 in middle dose and 0.1176 in highest doses respectively as against the control value of 0.0055 per metaphase. To observe these aberrations from lowest, middle and

highest doses of pesticide, 232, 242 and 102 metaphases were examined as against 361 metaphase of control, L.parsia. The aberrations per metaphase were 0.0158 in lowest dose, 0.0514 in middle dose and 0.1000 in highest doses respectively as against the control value of 0.0042 per metaphase after observed 252, 214, 200 metaphases as against 475 metaphase of control M.cephalus. The significant aberrations were observed in middle and highest dose whereas lowest dose did not induce significantly in both tested fishes.

The induction of micronuclei formation by phosphamidon was significantly obtained in middle and highest doses in L.parsia and M.cephalus (Table 22 & Fig.18 and Table 27 & Fig.23). The significant chromosome aberration were also observed from these concentration of these pesticide. This may be because 0.01 ppm of phosphamidon was not effective at genome level. In each dose 3,000 cell were examined to observe the micronuclei formation per cell of L.parsia and M.cephalus which were 0.0016 in lowest dose, 0.0036 in middle dose, 0.0086 in highest dose respectively as against the control value of 0.0006 per cell of L.parsia, and 0.0016 in lowest dose, 0.0026 in middle dose and 0.0120 in highest dose respectively as against the control value of 0.0004 per cell of M.cephalus.

Phosphamidon also induced the behavioural changes in both observed fishes (Table 33 & 34). Toor and Kaur (1974) reported that phosphamidon induced the behavioural changes in C. carpio communis. The same type of behavioural changes were also noticed in the present study. Galli et al.(1994) reported that organophosphorus pesticides are potent inhibitor of acetyl cholinesterase (AChE) enzyme and their inhibition activities were well established in vivo and in vitro in fishes (Weiss, 1958, 1959). Williams and Sova(1966) reported that the fishes inhabiting a river section down stream of a source of organic pollution also showed inhibition of AChE. In the present study, behavioural changes of L.parsia and M.cephalus may be due to inhibition of acetyl cholinesterase.

c) Dichlorvos 76% EC (Nuvan): The present work showed the chromosomal aberrations per metaphase were 0.0116 in lowest dose, 0.0779 in middle dose and 0.1125 in highest dose respectively as against the control value of 0.0055 per metaphase of L.parsia whereas in M.cephalus they were 0.0096 in lowest dose, 0.0210 in middle dose and 0.0292 in highest dose respectively as against the control value of 0.0042 per metaphase (Table 11 & Fig.7 and Table 16 & Fig.13). Significant aberrations of Nuvan were observed in middle and highest dose in L.parsia and M.cephalus. Chromosome aberration such as gaps, breaks, fragments, etc. were commonly observed in two species whereas isochromatid breaks and gaps were found only in L.parsia.

The frequency of micronuclei in L.parsia exposed to Nuvan for 96 hrs showed significant increase in highest and middle dose which were observed after 3,000 cells from each concentration (Table 23 and Fig.19). In the case of M.cephalus significant increase of micronuclei was also observed in middle and highest dose treatments. The occurrence of MN in L.parsia and M.cephalus were in dose response manner. This may be due to similar resistant of these species in regarding the MN formation. The occurrence of micronuclei per cell were 0.0020, 0.0030 and 0.0093 in lowest, middle and highest doses repectively as against the control value of 0.0006 per cell of L.parsia whereas in M.cephalus they were 0.0013 in lowest, 0.0076 in middle, and 0.0173 in highest dose (Table 28 and Fig.24).

Dichlorvos is an anticholinesterase agent with harmful effects on salmon (Ross, 1989) and on rainbow trout (Hoy et al., 1991). Its effect on cholinesterase in a dose response manner to the larvae of lobster, H.gammarus, herrings, C.herengus (Mc Henery et al., 1991) and to H.americanus, zooplankton and phytoplankton (Cusack and Johnson, 1990), and to crab, C.pagurus, C.maenas, lobster H.americanus and blue mussel, M.edulis (Egidius and Moster, 1987) were reported. In the present study, L.parsia and M.cephalus also showed behavioural changes. (Table 33 & 34). This may be due to the effect of dichlorvos on cholinesterase enzyme in dose response manner. Pal and Konar (1985b) reported that this pesticide also inhibited growth and reproduction of fishes. Therefore it is possible that the growth and reproduction of L.parsia and M.cephalus are inhibited by this pesticide.

d) Monocrotophos 36% SL (Nuvacron) : The effect of monocrotophos to L.parsia and M.cephalus showed significant increase of chromosome aberration in middle and highest dose (Table 12 & Fig.8 and Table 17 & Fig.13). In these two cases insignificant aberration was observed in lowest dose. This may be because this concentration of pesticide probably might not have reached the target molecule. Gaps, breaks and fragments were commonly observed in two test animals. But, centromeric separation and ring chromosome were observed in M.cephalus while isochromatid was observed in L.parsia. The dose response effect was observed in these two case of treatment.

The significant occurrence of micronuclei were observed in middle and highest concentration of pesticide in L.parsia and M. cephalus (Table 24 & Fig.20 and Table 29 & Fig.25). The dose response effect was also observed here. Micronuclei formation per cell and aberration per metaphase were found to be insignificant in both fishes at the treatment of 0.01 ppm. It is, therefore, revealed that this concentration of this pesticide was not effective to aberrate the chromosomes and micronuclei formation.

This pesticide also induced the behavioural changes of treated fishes, L.parsia and M.cephalus. (Table 33 & 34). The inhibition of acetyl cholinesterase by organophosphate pesticides in fishes were reported by Weiss (1961) and these enzymes are essential for normal neural transmission (Wild, 1975). In the present study,

the candidate species were also showed behavioural changes due to the action of monocrotophos. This may be due to the inhibition of acetyl cholinesterase by this pesticide in the treated fishes. Rajavarthini and Michael (1996) reported the effect of this pesticide on the suppression of the antibody response of O.mossambicus on dose dependent manner. So there is some possibility that this pesticide may also affect the immune system of candidate species.

e) Malathion 50% EC: The response of L.parsia and M.cephalus to Malathion was almost equal. Significant chromosome aberration ($Z \geqslant 1.96$) was observed only in highest dose (0.2 ppm) in L.parsia and M.cephalus and their aberration per metaphase were 0.0585 and 0.0227 respectively. Gaps, breaks, rings, fragments were observed from metaphase of the tested animals. But, centromeric separation was only observed in L.parsia (Table 13&18 and Fig.9&14). The same type aberrations were reported from malathion treated rainbow trout, Salmo gairdneri by Al-Sabti (1985 b).

The occurrence of micronuclei in the malathion treated L.parsia and M.cephalus showed significant increase only in highest dose (0.2 ppm) and their occurrence per cell were 0.0043 and 0.0060 respectively and the induction of micronuclei in the middle (0.1 ppm) and lower concentration (0.01 ppm) were not at significant level(Table 25 & 30 and Fig. 21&26).

Heddle et al. (1983) reported that the presence of micronuclei in mammalian somatic cells is recognized as a cytogenetic indicator of genotoxicity. The presnet study also demonstrated these as indicators of genotoxic effect in fishes. The frequency of aberrations was higher than that of micronucleated cells of fishes when same dose of X-rays was used (Manna and Some, 1982, Manna et al., 1985), because MN is formed due to limited type of anaphase chromosome aberrations like laggard and symmetricical changes and its durability is time bound (Hooftman and de Raat, 1982). The present study agrees with these observations.

Coppage and Matthews (1974), reported the mechanism of inhibition of acetyl cholinesterase by malathion. The noticeable behavioural changes in L.parsia and M.cephalus were observed in highest concentration (0.2 ppm) (Table 33 & 34). The behavioural changes of the candidate species may be due to action of malathion on the enzymes responsible for neural transmission.

In the present study, malathion induced at significant level ($Z \geqslant 1.96$) of chromosome aberration and micronuclei formation only in 0.2 ppm concentration (highest) whereas in the case of other pesticide 0.1 ppm (middle) concentration also induced the significant level of chromosome aberration and micronuclei formation while 0.01, 0.1, 0.2 ppm (lowest, middle, highest) concentration of methyl parathion exhibited significant level of chromosome aberration and micronuclei formation in L.parsia and M.cephalus. Thus it is revealed that malathion showed least toxicity in comparison to methyl parathion, phosphamidon, dichlorvos and monocrotophos while methyl parathion showed highest toxicity. The lesser toxicity of malathion may be due

to the ability of fish to hydrolyse malathion enzymatically to non toxic metabolite. Similar reasoning has been also given by Toor et al. (1973) for C.carpio; Lind Strom - Seppa and Oikari (1990) for S.gairdneri and Singh and Singh (1992) for H.fossilis.

Thus the present study revealed that the tested five organophosphorous pesticides induced chromosomal aberrations, micronuclei formation and behavioural changes in L.parisa and M.cephalus. Chromosomal aberration can also be used as an indicator for DNA damage in the cell. Such chromosomal aberration may leads to changes in the genetic components and concern has been expressed about the genetic consequence of pollution to fish population which is exposed to low level of pollution over a prolonged period. (Barker and Rackman, 1979). Kurelec (1993) suggested that genetic damage can cause the inhibition of growth, decrease the fecundity, and faster ageing. Therefore it is possible that these genetic damages may also decrease fecundity and faster ageing of L.parisa and M.cephalus.

Atlantic salmon Salmo salar exhibited aggressive and territorial behaviour at 1 ppm concentration of fenitrothion an organophosphate pesticide (Bull, 1971; Symons, 1972), became more vulnerable to predation (Hatfield and Anderson, 1972) and behavioural changes of P.pugio due to anticholinesterase activity of methyl parathion which induced impairments of antipredatory behaviour and it can ultimately impair the survivability of this species (Farr, 1977) and organochlorine also induced greater predation on grass shrimp P.pujo

(Tagatz, 1976). In the present study, as the fishes exhibited the same type of behavioural changes and decreased physical stamina, it may also leads to impairment of antipredatory behaviour of L.parsia and M.cephalus. Paine (1966) also reported that impairment of antipredatory activity in a community can alter species diversity and community structure.

Many studies revealed that organophosphate pesticides affected the reproduction of fishes. Wester et.al. (1985); Dethlefsen and Tiews (1985) reported the acute effect of pesticides resulting in mass mortality or changes in survival, growth and reproduction. The inhibition activity of pesticides on the reproductive parameters such as steroidogenic enzyme in C.carpio communis (Kapur et al., 1978), in C. batrachus (Singh and Singh, 1985), ovarian steroidogenesis in C.batrachus, in C.punctatus (Inbaraj and Hyder, 1988), vitellogenesis in S.gairdneri (Chen et al., 1986), ovarian developments in S.fontinalis (Macek, 1969), ovulation in O.latipes (Hirose, 1975), gonadotrophin secretion in H.fossilis (Singh and Singh, 1980, a,b, 1981), and inhibition of ovarian steroidogenesis in H.fossilis (Singh and Singh, 1992) . Thus from the above mentioned reports, it is revealed that organophosphate pesticides are affecting the reproductive parameters such as delayed sexual maturity, reduced reproductive efficiency, hatchability of eggs, regression of gonads, etc. in fishes. Therefore in the present study also, there is some possibility that the effects of these pesticides may leads to reproductive parameters of L.parsia and M.cephalus.

There is some circumstantial evidence linking sub-lethal exposure to pollutants and disease of fish (Sinderman, 1979). Stephanie Pain (1989) and Stephen Mills (1989) reported that dichlorvos caused cataracts in salmon. Fish in chemically polluted area (Krabill *et al.*, 1977; Malins *et al.*, 1984, 1985) or exposed under controlled laboratory conditions (Hendricks *et al.*, 1985) exhibit a high frequency of neoplasia, indicating a possible association of pollutant with tumor development. Ostrander *et al.* (1992) reported retinoblastoma in Japanese medaka, *O.latipes* after exposure of methylazoxymethanol acetate. Kocan *et al.* (1985a) reported that B(a)P and MNNG induced anaphase aberration, pathological changes, spinal deformities and renal hypoplasia in *S.gairdneri*. Liguori and Landolt (1985) reported that anaphase aberration, hatching and developmental defects rainbow trout *S.gairdneri* after exposure to N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG). In this work, difference in pigmentation and unilateral hypoplasia were noticed in the MNNG exposed fishes. They have also reported that it is possible to correlate the cytological responses with pathological conditions in the organisms. This correlation enlights the use of short term assay as an indication of environmental pollution. The results of the present study vividly showed that the pesticides like methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion induced genetic damage in the form of chromosome aberration, and micronuclei formation and there is some possibility that the toxic effect of pesticide may also leads to pathological condition in *L.parsia* and *M.cephalus*.

C O N C L U S I O N

The present study dealt with the genotoxic effect of pesticides viz. methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion on L.parsia and M.cephalus, and the following important conclusions were drawn.

1. Lates calcarifer, Scatophagus argus, Liza parsia and Mugil cephalus were screened. Among these later two species were found to be suitable for this study.

2. The method developed for in vivo chromosome preparation from the procedure followed by earlier workers has been found to be most suitable for the candidate species viz. Liza parsia and Mugil cephalus and has given best results.

3. Gill, liver and kidney tissues were used for chromosome preparation. Of these, gill tissue gave best spread of metaphase in air drying method.

4. The present study has confirmed first time the diploid number (2n) of chromosome as 48 in the test animals, L.parsia and M.cephalus in the west coast of India. All chromosomes were acrocentric type and its size ranged from 4.800 to 2.125 μ, relative length 5.332 to 2.361% in L.parsia, and in M.cephalus the size ranged from 5.5280 to 2.2505 μ and relative length 6.402 to 2.606%.

5. Based on earlier worker's reports, a modified method was developed for chromosome preparation and micronuclei studies.

6. The pesticides used in the studies induced chromosome aberration in both species at varying levels. Exposure of L.parsia to 0.2 ppm (highest) concentration of methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion induced chromosome aberration at significant level ($Z \geqslant 1.96$) and the aberration per metaphase were 0.1200, 0.1176, 0.1125, 0.0923 and 0.0585 respectively, and that of M.cephalus were 0.1033, 0.1000, 0.0292, 0.0436 and 0.0227 respectively. In the case of middle concentration (0.1 ppm) of pesticides, significant level of chromosome aberration were observed in all pesticides except malathion in both species, whereas in the lowest concentration (0.01) only methyl parathion showed significant aberration ($Z \geqslant 1.96$) in L.parsia and M.cephalus, and their aberration per metaphase were 0.0346 and 0.0377 respectively. But, malathion at its middle (0.1 ppm) and lowest (0.01 ppm) concentrations did not exhibit significant chromosome aberration in both species. It was, therefore, concluded that methyl parathion has maximum genotoxic effect on these two species while malathion has the least genotoxicity.

7. The significant occurrence of micronuclei was observed in highest concentration (0.2ppm) of all pesticides viz. methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion and their induction of micronuclei per cells were 0.0303, 0.0086, 0.0093, 0.0180 and 0.0043 respectively in L.parsia whereas in the case of M.cephalus the occurrence per cells were 0.0346, 0.0120, 0.0173, 0.0226 and 0.0060, respectively. In the middle concentration, all pesticides except malathion showed significant ($Z \geqslant 1.96$) occurrence of micronuclei in L parsia and M.cephalus. At the lowest

concentration (0.01 ppm), only methyl parathion induced micronuclei at significant level in L.parsia and M.cephalus and their occurrence were 0.0046 and 0.0060 per cells respectively. Therefore, these studies have also concluded that among the pesticides, methyl parathion has been found to be of maximum genotoxic effect, while, malathion was least genotoxicity to L.parsia and M.cephalus.

8. Observations on the physical reaction of treated L.parsia and M.cephalus also revealed that these five pesticides altered the normal behaviour of tested fishes.

S U M M A R Y

1. The topic of research has been introduced highlighting the scope of the work and its relevance to the present concept of genotoxicity studies on fishes.

2. An extensive review of literature has been provided on fish chromosome studies on a global level. The fish chromosome studies are reviewed right from the beginning. A historical resume on fish genotoxicity with special reference to chromosome aberrations and micronuclei studies are also given.

3. Standardisation of methods for chromosome and micronuclei studies have been furnished.

4. The screening of test animals, in vivo chromosome preparation, diploid number confirmation and karyotype analysis are described.

5. Base line chromosome aberrations of 0.0055 and 0.0042 per metaphase and rate of micronuclei formation at 0.0006 and 0.0004 per cell are reported for first time in L.parsia and M.cephalus from the west coast of India.

6. Methyl parathion, phosphamidon, dichlorvos, monocrotophos and malathion have been used to study the genotoxicity on the above species based on various end points like chromosome aberrations and micronuclei formation.

7. Various type of chromosome aberrations like gaps, breaks, fragments, rings, isochromatid gaps and breaks, centromeric separations and fusions were observed in treated fishes (L.parsia and M.cephalus).

8. Rate of chromosome aberration per metaphase and micronuclei formation per cell were dose dependent with all tested pesticides in L.parsia and M.cephalus.

9. Among the tested pesticides, methyl parathion exhibited maximum genotoxic effects while malathion showed minimum genotoxicity.

10. Normal behaviour of L.parsia and M.cephalus were changed due to the effects of these pesticides and their intensities were in dose response manner.

11. The detailed discussion on the screening of test animals, chromosome preparation, diploid numbers, analysis of karyotype, micronuclei formation, base line and induced chromosome aberrations in L.parsia and M.cephalus has been provided.

12. Eight important conclusions were also drawn on the basis of results of the present study.

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