INVESTIGATION ON MITOTIC DIVISION OF DIFFERENT TYPES OF TISSUES IN SILVER CARP *HYPOPHTHALMICHTHYS MOLITRIX* (VALENCIENNES IN CUVIER AND VALENCIENNES, 1844) AND SILVER BARB *BARBONYMUS GONIOTUS* (BLEEKER, 1850)*

Thidar Aung[1], Thawdar Aung[2]and Win Win Mar[3]

Abstract

The silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* belonging to family Cyprinidae were collected from Thatyetkone Fisheries Station, Mandalay Region, during January to August, 2022 to evaluate the mitotic check of cellular process. Various colchicine concentrations (CC) were injected below the pelvic fins depend on the fish weight (1 ml/100g). Liver, oral cells, kidney, heart, gill filaments and blood cells were extracted and treated with hypotonic solution (HS) 0.56 % KCL for various durations. In silver carp, the highest frequencies of metaphase stage 100 % (n=22) in kidney treated with CC 0.50 % for 5 hrs with HS for 1 hr, 81.25 % (n=13) in liver treated with CC for 3 hrs by exposing HS for 45 mins, 75.00 % (n=18) in heart with CC for 4 hrs and HS for 1 hr, and the lowest 47.83 % (n=11) in kidney with CC for 5 hrs with HS for 1 hr 30 mins. In silver barb, the highest frequencies of metaphase stage were 88.14 % (n=52) in gill filaments treated with CC 0.10 % for 2 hrs with HS for 10 mins, 79.17 % (n=57) in kidney treated with CC 0.10 % for 2 hrs with HS for 30 mins, 88.10 % (n=37) in gill filaments treated with CC 0.05 % for 5 hrs by exposing HS for 1 hr, 30.77 % (n=32) in kidney with CC for 6 hrs and HS for 1 hr, 12.50 % (n=8) in blood cells with CC for 4 hrs 45 mins with HS for 1 hr and the lowest 4.94 % (n=4) in oral cells with CC 0.30 % for 2 hrs with HS for 2 hrs 45 mins. These results will provide the foundation of genetic assessment in mitotic cell division of freshwater fishes.

Key words: Fishes, mitotic division, colchicine, KCL, frequencies

Introduction

The aquaculture is the second sector of economic income for sustainable development of human resource management. Among the fishes, silver carp *Hypophthalmichthys molitrix* (Valenciennes in Cuvier and Valenciennes, 1844) is native to China, Mongolia and Russian Federation, and the silver barb *Barbonymus gonionotus* (Bleeker, 1850) locally known as (Nga-khone-ma-kyee) is distributed around from Viet Nam, the Mekong basin in Lao PDR, Cambodia and Thailand (Froese and Pauly, 2022 and Integrated Taxonomic Information System, ITIS, 2022).

Mitosis is a process of nuclear division in which the replicated DNA molecules of each chromosome are faithfully segregated into two nuclei and maintains the identical chromosome numbers and generates haploid or diploid cells (Iwasa and Marshall, 2018). The cytogenetic studies on silver carp *Hypophthalmichthys molitrix* locally known as (Nga-gyin-phyu) and silver barb *Barbonymus gonionotus* locally known as (Nga-khone-ma-kyee) from Thatyetkone Fisheries Station are concerning with these information's, the different methodological approaches on cytogenetic studies have been limited in various research areas. The objectives of this study were to designate the mitotic inhibitors of various cells and to investigate the frequency of mitotic cells in these fishes during mitotic divisions.

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[1] Department of Zoology, University of Mandalay
[2] Department of Zoology, University of Mandalay
[3] Department of Zoology, University of Mandalay

Materials and Methods

The present research work was conducted at Laboratory, Department of Zoology, University of Mandalay. The study period was from January 2022 to August 2022. Twenty fish samples for each species were collected from Thatyetkone Fisheries Station which is situated between 21° 59' 28.53" N, and 96° 7' 44.60" E, Patheingyi Township, Mandalay Region. The collected fishes were cultured at Laboratory, Department of Zoology, University of Mandalay. The commercial pellets were fed twice a day. The water was changed twice a week and reared in well-aerated aquarium.

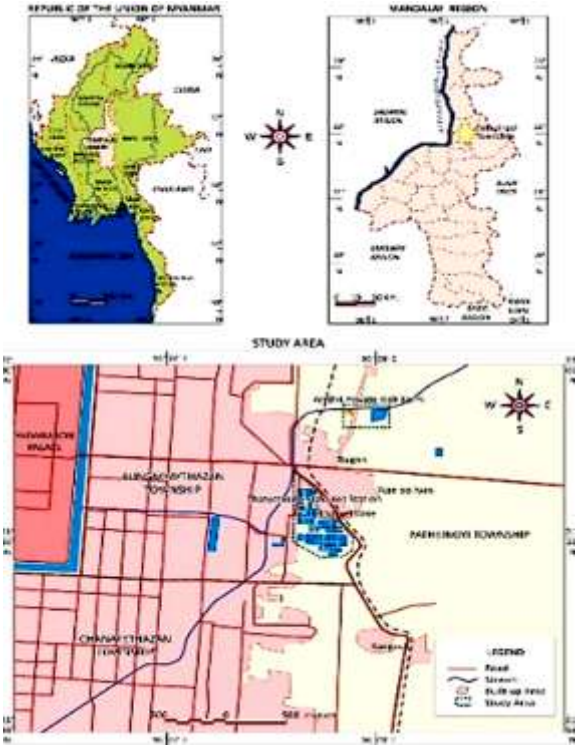


Figure.1. Study map of Thatyetkone Fisheries Station, Mandalay Region (Source: UTM)

Identification of species

The species identification was followed by Talwar and Jhingran (1991), and Integrated Taxonomic Information System (ITIS), (2022).

Injection technique

The total length and standard length of fish were measured by a plastic ruler to the nearest 0.1 cm, and their weight were recorded by digital balance to the nearest 0.01 g. The concentrations of colchicine solutions such as 0.10 % for 1 hr 30 mins and 2 hrs; 0.30 % for 2 hrs and 0.50 % for 3 hrs, 4 hrs, 4 hrs 45 mins, 5 hrs and 6 hrs were used, and injected at the base of pelvic fin that depend on the fish weight (1 ml/100g) (Plate 1, 2 B and C).

Plate 1. Injection technique

Collection of blood and tissues

The blood was collected by using a syringe, diluted with 0.56 % KCL solution and sacrificed with 10 % formaldehyde solution. Different tissues such as liver, heart, kidney, oral cells and gill filaments were also extracted from each fish (Plate 2 D).

Extraction of cells

Each tissue sample was kept in block-cup filled with 0.56 % KCL solution, minced thoroughly with glass rod and incubated for 10 mins, 20 mins, 30 mins, 45 mins, 1 hr, 1 hr 30 mins and 2 hrs 45 mins, and transferred to 6 cm glass test tube and mixed homogeneously by a Vortex mixer and centrifuged at 2000 rpm for 5 mins. The supernatant was discarded by using a pipette and the pellets were treated with 3 methanol:1 acetic acid for 15 mins. The process was repeated twice again (Plate 2 E, F, G and H).

Preparation of slide, Giemsa stain and identification

One or two drops of samples was placed onto the warm slide with a far distance, dehydrated at room temperature, stained with undiluted Giemsa stain for 10 mins, washed under running tap water, and then dried at room temperature. The permanent slides were prepared by dropping one or two drops of immersion oil, coated with Canada balsam, and checked under biological microscope (x1000) with attached camera (Plate 2 I, J, K and L).

Statistical analysis

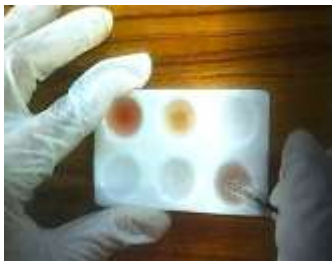
The frequency of chromosomal configurations in different stages of cells from various tissues of silver carp and silver barb were generated by Microsoft Excel, 2010.

A. Fish rearing in aquarium

B. Weighting the fish

C. Injection of the fish

D. Extraction of blood

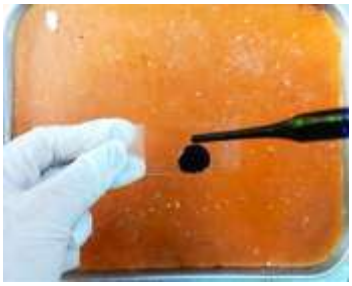


E. Mechanical dissociation of tissues

F. Homogenization of tissues

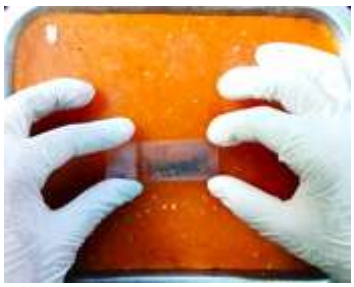
G. Centrifugation of pellets

H. Discarding the supernatant from the test tube



I. Staining on the slides

J. Washing under tap water



K. Coating with cover slip

L. Examination of microscope slide

Plate 2. Preparation of cytological process from fish

Results

The frequency distribution of mitotic division in different tissues and blood cells of silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* were investigated by treating with three types of colchicine concentrations 0.10 %, 0.30 % and 0.50 % with various durations 1 hr 30 mins, 2 hrs, 3 hrs, 4 hrs, 4 hrs 45 mins, 5 hrs and 6 hrs, and then exposing with 0.56 % KCL solution for durations such as 10 mins, 20 mins, 30 mins, 45 mins, 1 hr, 1 hr 30 mins and 2 hrs 45 mins, respectively. Different types of tissues generated the different stages of chromosomal configuration containing with various frequencies of each stage (Plate 3).

Percent and frequency distribution in silver carp *Hypophthyalmichthys molitrix*

The colchicine concentration 0.50 % for duration 3 hrs with 0.56 % KCL for 45 mins generated the highest frequency of blood (100.00 %, n=7) at interphase stage followed by kidney (82.35 %, n=28), liver (54.76 %, n=23), oral cells (42.86 %, n=12), gill filaments (50.00 %, n=12) and heart (20.83 %, n = 5) for 4 hrs colchicine solution with 0.56 % KCL for 1 hr.

The highest frequency of blood cells (35.48 %, n=11) and the lowest frequency of liver (2.38 %, n=1) produced the prophase stage at 4 hrs colchicine treated with 0.56 % KCL for 1 hr. The highest prophase frequencies (oral cells, 33.33 %, n=12 and kidney, 31.88 %, n=22) were observed in colchicine solution (CS) for 3 hrs with 0.56 % KCL duration for 45 mins. The frequency of prophase stage in heart (25.00 %, n=4) was highest in CS 5 hrs with KCL duration 1 hr and the highest prophase frequency for liver (60.87 %, n=42) was observed in CS 5 hrs with KCL for 1 hr 30 mins.

The highest frequency of metaphase stage (75.00 %, n=18) in heart by treating with CS for 4 hrs with KCL for 1 hr. However, CS for 5 hrs generated the metaphase stage in oral cells (67.39 %, n=31), kidney (100.00 %, n=22), liver (80.00 %, n=44), heart (62.50 %, n=10) and gill (72.27 %, n=17) by exposing KCL for 1 hr (Fig. 2, 3, 4, 5).

Percent and frequency distribution in silver barb *(Barbonymus gonionotus)*

The highest metaphase stage of chromosomes 88.14 % (n=52) in gill filaments, followed by 74.36 % (n=29) in blood cells,62.63 % (n=62) in kidney by treating with 0.10 % CS for 2 hrs with 0.56 % KCL for 10 mins. The highest frequency of interphase stage 79.22 % (n=61) from oral cells was observed at 0.10 % CS for 2 hrs and 0.56 % KCL for 30 mins. The CS for 2 hrs with 0.56 % KCL for 10 mins generated the prophase stage 21.21 % (n=21) (Fig. 6,7).

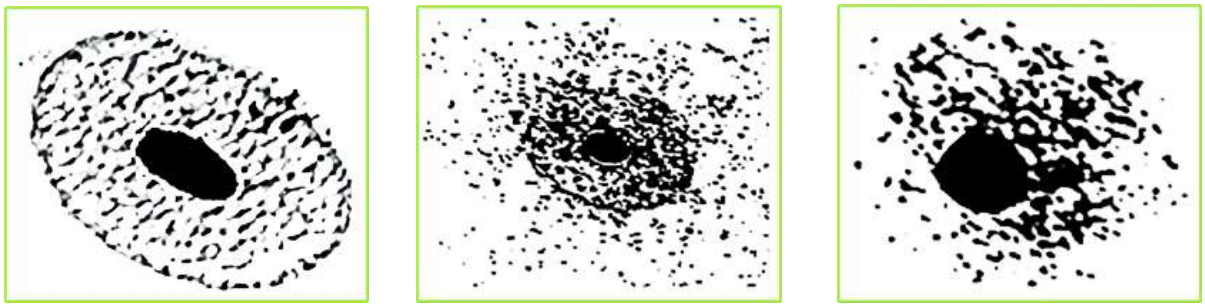
The highest frequency of interphase stages was observed in oral cells 93.82 % (n=76) and 100.00 % (n=95) in liver cells by treating with 0.30 % CS for 2 hrs with 0.56 % KCL for 2 hrs 45 mins. The lowest frequency of prophase stage 1.23 % (n=1) and metaphase stage (4.94 %, n=4) were found in oral cells (Fig. 8).

The liver cells generated the highest frequency of interphase stage 100.00 % (n=98) and prophase stage (kidney) 17.07 % (n=7) and metaphase stage from blood cells 12.50 % (n=8) by treating with 0.50 % CS for 4 hrs 45 mins with 0.56 % KCL for 1 hr. The mitotic stages of cells were not observed in oral cells and heart (Fig. 9).

The highest metaphase stage 88.10 % (n=37) in gill filaments followed by oral cells 80.36 % (n=45); prophase stage 26.22 % (n=16) in kidney cells and interphase stage 55.77 % (n=29) in heart cells by treating with 0.50 % CS for 5 hrs with 0.56 % KCL for 1 hr. The lowest prophase stage 5.36 % (n=3) was found in oral cells. Among these tissue cells, the mitotic division was not observed in blood cells (Fig. 10).

The mitotic cells division were not observed in blood cells and oral cells by treating with 0.50 % CS for 6 hrs with 0.56 % KCL for 1 hr. However, the highest frequency of interphase

stage 75.93 % (n=82) in gill filaments followed by prophase stage 59.26 % (n=16) in liver cells and metaphase stage 30.77 % (n=32) in kidney cells (Fig. 11).



A. Early interphase stage B. Middle interphase stage C. Late interphase stage

D. Early prophase stage E. Middle prophase stage F. Late prophase stage

G. Early metaphase stage H. Middle metaphase stage I. Late metaphase stage

Plate 3 The arrested stages of chromosomal configurations in mitotic cell division

Figure.2. Effect of colchicine concentration 0.50 % for 3 hrs with 0.56 % KCL for duration 45 mins on the mitotic division of different tissues *Hypophthalmichthys molitrix* (1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver)

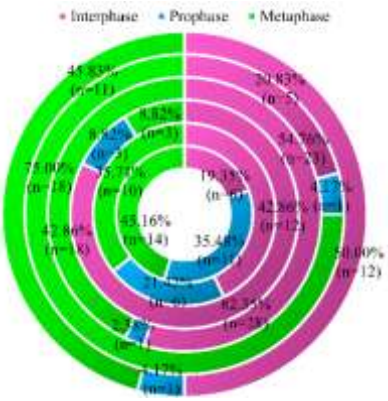


Figure.3. Effect of colchicine concentration 0.50 % for 4 hrs with 0.56 % KCL for duration 1 hr on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring- blood cells; 2nd ring– oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- heart; 6th ring-gill filaments)

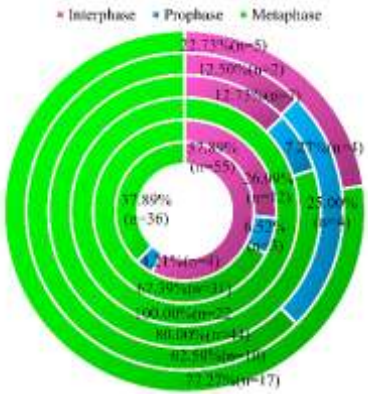


Figure.4. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring-blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- heart; 6th ring-gill filaments)

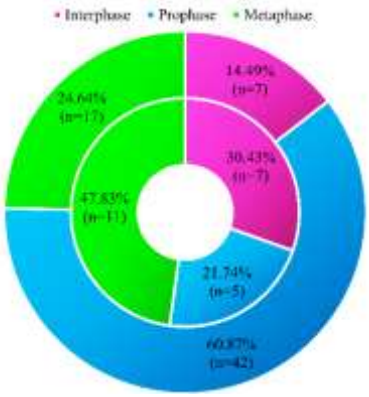


Figure.5. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr 30 mins on the mitotic division of different tissues in *Hypophthalmichthys molitrix* (1st ring- kidney; 2nd ring- liver)

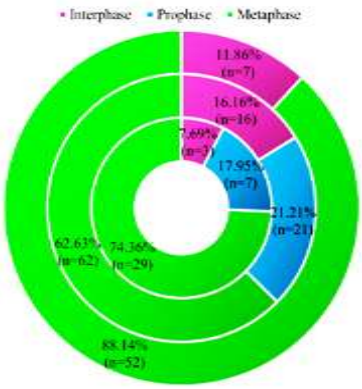


Figure.6. Effect of colchicine concentration 0.10 % for 2 hrs with 0.56 % KCL for 10 mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- blood cells; 2nd ring- kidney; 3rd ring- gill filaments)

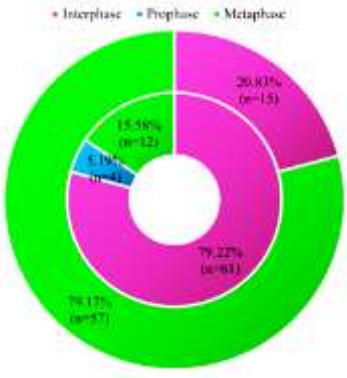


Figure.7. Effect of colchicine concentration 0.10 % for 2 hrs with 0.56 % KCL for 30 mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kidney)

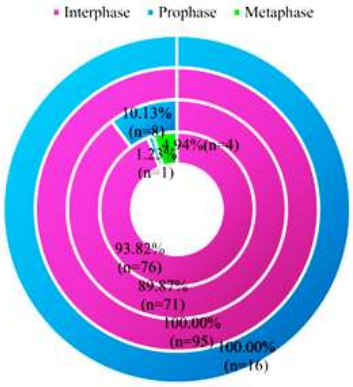


Figure.8. Effect of colchicine concentration 0.30 % for 2 hrs with 0.56 % KCL for 2 hrs 45mins on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kideny; 3rd ring- liver; 4th ring– gill filaments)

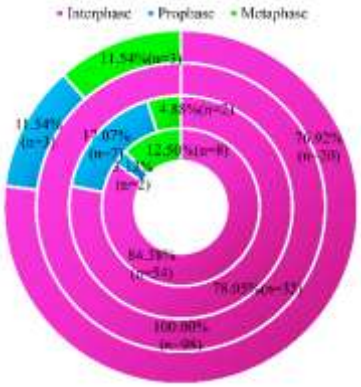


Figure.9 . Effect of colchicine concentration 0.50 % for 4 hr 45 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st - blood cells; 2nd ring- kidney; 3rd ring- liver; 4th ring- gill filaments)

Figure.10. Effect of colchicine concentration 0.50 % for 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- oral cells; 2nd ring- kidney; 3rd ring- liver; 4th ring- heart; 5th ring- gill filaments)

Figure.11. Effect of colchicine concentration 0.50 % for 6 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues in *Barbonymus gonionotus* (1st ring- kidney; 2nd ring- liver; 3rd ring- heart; 4th ring - gill filaments)

Discussion

The mitotic division of silver carp *Hypophthalmichthys molitrix* and silver barb *Barbonymus gonionotus* were investigated by injection of different concentration of colchicine solutions at the base of pelvic fin of fish depending on the fish weight 1 ml / 100 g. Three different mitotic stages viz interphase, prophase and metaphase stages were observed in which interphase and metaphase stages were more distributed than prophase stages. Every treatment generates these stages that are depending on the colchicine concentration and hypotonic treatment with different durations.

Each stage of mitotic division is designated to the chromosomal configuration in each cell spread. In silver carp, late interphase stage of cells was more observed in colchicine concentration 0.50 % for 3 hrs with 0.56 % KCL for 45 mins. The early metaphase stage from different tissues reveals 4 hrs with 0.56 % hypotonic solution for 1 hr. When fish were treated with colchicine solution (CS) 5 hrs injection with 1 hr fixation of hypotonic solution, the early and middle metaphase stages of chromosomes were observed. The complete sets of metaphase

chromosomal configurations were observed in 5 hrs colchicine treatment with 0.56 % KCL for 1 hr 30 mins. In silver carp, the best metaphase stage was observed in liver cells 81.25 % (n=13) and 80.00 % (n=44) as well as in kidney cells 100 % (n=22) than other different stages of mitotic cells division. This is the optimum treatment for desired condensation degree of metaphase chromosomes.

These treatments were also generated the best metaphase stage in oral cells 80.36 % (n=45) and gill filaments 88.10 % (n=37) treated with 0.50 % CS for 5 hrs with KCL for 1 hr followed by kidney cells 30.77 % (n=32) in silver barb for 6 hrs with KCL for 1 hr. The condensed chromosomal configuration was observed in 0.50 % colchicine concentration for 4 hrs 45 mins duration with 0.56 % hypotonic solution for 1 hr indicating the optimal stage of metaphase check point of cells in silver barb. The middle stage of metaphase stage was observed in 5 hrs injection and hypotonic treatment duration for 1 hr. The complete metaphase stages were observed in colchicine treatment for 6 hrs and hypotonic solution treatment for 1 hr.

The silver barbs were injected with colchicine concentration 0.10 % for a duration 1 hr 30 mins and the extracted cells were treated with 0.56 % hypotonic solution for 10 mins. The insufficient amount of solution unable to disrupt the mitotic spindle formation. Although the cells become reached on the metaphase stage in mitotic division, the complete metaphase stages of cells were not observed. Thus, this treatment could not be resolved to get the optimal checkpoint of mitotic cell division. The early metaphase stage of chromosomes was observed in 0.10 % colchicine treatment 2 hrs with hypotonic treatment duration for 10 mins and 30 mins. The longer exposure to the hypotonic solution leads to the random spreading of chromosomes on the slides. When injection treatment of 0.30 % colchicine solution was for 2 hrs and 2 hrs 45 mins in hypotonic solution, the early synchronized metaphase stages of cells were randomly spread due to overtreatment duration of hypotonic solution.

These results were correspondence to Mark (2000) who reported that the longer culture is exposed to colchicine, the greater the potential number of arrested metaphases. In addition, they reported that undertreatment of hypotonic solution leads to inadequate spreading and unable to distinguish individual chromosomes as well as overtreatment in hypotonic solution leads to overspreading with rupture of the cell membrane and random loss of chromosomes (Dorathy and Mokhasi, 2013).

In this study noted that the duration of Carnoyl's fixative for 15 mins was the optimal condition for the preservation and suspension of the cells in silver carp and silver barb. The good shape of miotic chromosome spreads were observed by using pre-warmed slides and stained with undiluted Giemsa stain for 10 mins. The overstaining leads to count difficultly and identify the chromosomal configurations in every step of mitotic division. Giemsa stain was effective for getting the good shape of chromosomes in prepared slides.

Conclusion

The different degree of chromosomal configuration such as interphase stage (early, middle, late) and prophase (early, middle, late) and metaphase stage (early, middle, late) were observed in various tissues of silver carp and silver barb. The best optimization of mitotic cell division was 0.50 % colchicine solution for duration 5 hrs with 0.56 % KCL for 1 hr in silver barb but 4 hrs with 0.56 % KCL for 1 hr were investigated as the optimal duration in silver carp for karyological analysis. The present results provide the basic information of chromosomal studies on designated fish before identification of karyotypic analysis of these fish. In addition, the mechanical assessment and chemical solutions on treatment of different cells generated various kinds of mitotic division stages.

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