Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi
Menba Journal of Fisheries Faculty
ISSN 2147-2254 | e-ISSN: 2667-8659

Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2024; 10 (3): 113-118 Araştırma Makalesi/Research Article

Effects of Colchicine and Trypsin Treatments on Metaphase Chromosome Spreading from Larvae and Different Tissues of Adult Female and Male Nile Tilapia

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Article Info:
Received:
26/08/2024
Accepted:
09/11/2024

Keywords :
- Metaphase spread
- Chromosome
- Colchicine
- Trypsin
- Nile Tilapia (O. niloticus)

Abstract

Assessment of the results of different biotechnological methods in aquaculture such as ploidy, gynogenesis, and androgenesis are needed classical cytogenetical techniques like karyotype and chromosome number. Achieving well-distributed metaphase chromosome spreads is a preeminent step for the cytogenetical techniques. The aim of this study was to evaluate the effects of colchicine and trypsin treatments on the yield of countable metaphase chromosome spreads. With this aim, chromosome preparations from gill, spleen, kidney, and caudal fin tissues of adult females and males Nile tilapia (*Oreochromis niloticus*) as well as from whole body of their larvae were evaluated. Based on the previous studies reviewed so far, this is the first systematic and extensive study indicated that the effects of colchicine and trypsin on metaphase chromosome spreads regarding gender differences and larvae. In the chromosome preparations, it was designated four treatments as "Trypsin (-) Colchicine (-)", "Trypsin (-) Colchicine (+)", "Trypsin (+) Colchicine (-)", and "Trypsin (+) Colchicine (+)". Effects of colchicine and trypsin in terms of their usage in chromosome preparations were assessed by determining the frequencies of metaphase cells. For this purpose, a metaphase index was used to reveal differences in detection of metaphase cells among the treatments. The highest metaphase index values were calculated in the Trypsin (+) Colchicine (+) treatment group of all tissues. Also, the scores of metaphase index found in spleen and kidney tissues were greater than those found in gill and fin tissues. The present study has also shown that metaphase chromosome spreads could be obtained without colchicine or trypsin treatments.

Plastics, one of the most common materials polluting our seas, are now a serious global problem. These plastics persist in our environment for a long time and gradually turn into much smaller particles that we call microplastics (MPs). In this study, the MPs profile of sand and seawater samples taken from 6 different stations from the coasts of Ordu Province was analysed in detail. As a result of MPs and µ FTIR spectroscopic analyses, the presence of MPs in sand and seawater samples was determined and their characterisation, abundance and distribution characteristics were revealed. In this study, 291.11 items kg-1 MPs was found in sediment samples and 0.263 items L-1 MPs in water samples. A total of 420 MP fragments were detected from seawater and sand samples on the coasts of Ordu Province and analysed for colour, shape, size and species. Fibre and film type MPs fragments were found the most and it was determined that these fragments were generally blue and transparent in colour. It was observed that MPs were commonly in the size range of 0-50µm (50.71%) and the detected MPs were not larger than 800 µm. Most of the MPs observed were polyethylene (56%), followed by polypropylene (19%), polystyrene (15%), polyvinyl acetate (7%) and polytylene tereftelate (3%). In conclusion, MPs pose serious threats to human health and the environment, and it is recommended that waste generation should be reduced, necessary precautions should be taken, monitoring studies should be carried out and necessary removal methods should be applied in order to reduce the risk caused by wastes released into the seas.

Atıf bilgisi/Cite as: İnanan, B. E. (2024). Effects of Colchicine and Trypsin Treatments on Metaphase Chromosome Spreading from Larvae and Different Tissues of Adult Female and Male Nile Tilapia. Menba Journal of Fisheries Faculty. 10 (3), 113-118. DOI: 10.58626/menba.1538957.

INTRODUCTION

Like in mammalian species, cytogenetical studies on fish have a remarkable importance in different research fields such as evolutionary, taxonomical, and mutagenesis studies as well as aquaculture and quality control (Foresti et al. 1993). Especially consideration and evaluation of results of some biotechnological methods in aquaculture related to fish stock management such as ploidy, gynogenesis, and androgenesis are needed cytogenetic techniques (Demirok and Ünlü 2001; Pradeep et al. 2011). Obtaining well-distributed metaphase chromosomes is the first and critical step for cytogenetical techniques (Deng et al. 2003).

Although some similarities exist in their main steps, different techniques such as squash and chopping techniques have been used for an optimum metaphase chromosome spreading (Foresti et al. 1993; Pradeep et al. 2011; Mukti et al. 2016). Also, these techniques are deployed *in vivo* (i.e. application of a technique to living individuals and colchicine treatments via injection or immersion) and/or *in vitro* (i.e. application of a technique to dissected tissues). On the other hand, these techniques vary in treatments of colchicine and trypsin (Chourrout and Happe 1986; Henegariu et al. 2001; Harvey et al. 2002; Supiwong et al. 2013).

The aim of this study was to evaluate the effects of colchicine and trypsin treatments on the yield of increase countable metaphase chromosome spreads. With this aim, there was carried out the same technique for chromosome preparation from gill, spleen, kidney, and caudal fin tissues of adult females and males Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). Moreover, the same technique was applied to Nile tilapia larvae. In reviewing the literature, this is the first systematic and extensive study indicated that the effects of colchicine and trypsin on metaphase chromosomes spreading considering gender differences and larvae.

MATERIALS AND METHODS

Gill, spleen, kidney, and caudal fin tissues from three adult females and three adult males Nile tilapia have been sampled for chromosome preparations. Also, three of two-day-old larvae of Nile tilapia were sampled as the whole body. All fish were deeply anesthetized with clove oil before the sampling. Egg sacs and debris of larvae have been delicately removed using scalpels. The freshly removed tissues were cut into 1-2 mm^3 in size after the dissections of adults. After sampling, the tissues were stored in Carnoy's solution and preparations were made within a month (Hussain and Mcandrew 1994)

In the chromosome preparations, it was designated four treatments as "Trypsin (-) Colchicine (-)", "Trypsin (-) Colchicine (+)", "Trypsin (+) Colchicine (-)", and "Trypsin (+) Colchicine (+)". Except for usage of these chemicals, special care has been taken to ensure that each preparation is subjected to the same procedure. The chemicals were purchased from Sigma-Aldrich Chemical Company.

Tissues were washed in Petri dishes containing Ringer's solution to remove any adhering fat or blood. Tissues were chopped and minced in the same solution by a scalpel at room temperature. After the chopping tissues, 0.1 % trypsin and/or 0.03 % colchicine (w/v of Ringer's solution) were/was applied or not to form the treatments mentioned above. The suspensions placed into conical centrifuge tubes were incubated at 37 °C for 15 minutes. The suspensions were then centrifuged at 1000 rpm for 7 min. After removing the supernatants, the suspensions were shaken gently and added 0.075 M of KCl. After stirring lightly, the tubes containing the suspensions were incubated at 37 °C for 40 minutes. Subsequently, the fixation process was started with Carnoy's solution. The suspensions were gradually treated with Carnoy's solution. After that, the concentrated suspensions were dropped onto cleaned and heated slides the day before. After allowing them to dry, the preparations were rinse with acetone and dried again. The preparations were then stained with freshly prepared 10% Giemsa stain at exposure times of 20 min. After the staining, the preparations were plunge in xylene. After the xylene wash, the preparations were rinsed in distilled water, and afterwards dried and mounted with DPX (Yamazaki et al. 1981; Pradeep et al. 2011).

Five preparations were obtained from each sampling and were examined under Zeiss Axioscope 5 (Carl Zeiss Microscopy, Jena, Germany) microscope under 400× and imaged using the Zeiss Zen 3.1 (blue edition) program. Around 1000 cells each tissue preparate obtain were evaluated. Effects of colchicine and trypsin in terms of their usage the procedure on obtaining metaphase cells were evaluated by determining the frequencies of metaphase cells. For this purpose, a metaphase index formulated as total number of cells/number of metaphases were used to reveal differences in detected metaphase cells among the treatments (modified from Bertão and Aguiar-Perecin, 2002). Enumeration of cells was carried out with the assistance of imageJ software (Schneider et al. 2012)

The data were all expressed as means ± standard deviation and analyzed by SPSS 23.0 software. ANOVA following Tukey's test was used to determine whether results from each treatment were significantly different. The significance level was set a priori at $p < 0.05$.

RESULTS AND DISCUSSION

The present results demonstrated that metaphase chromosome spreads could be obtained from different tissues of adult individuals and larvae of Nile tilapia by the chopping method (Figure 1). When reviewing relevant literature on fish, the first application of

successful chopping method was observed in embryos of chum salmon (*Oncorhynchus keta*) and rainbow trout (*O. mykiss*), using with trypsin treatment (Yamazaki et al. 1981). On the other hand, previous studies have focused on arresting metaphase chromosomes using different techniques from various tissues of tilapia species. Foresti et al. (1993) obtained cells in metaphase of Nile tilapia kidney, gills and fin tissues by a modified method with or without colchicine treatment. Also, peripheral blood leukocytes from *O. karongae* and *O. niloticus*×*O. karongae* hybrid could be used for arresting metaphase chromosome spreads (Harvey et al. 2002). Besides leukocytes, metaphase chromosome spreads could be successfully attained by squash technique from testis Nile tilapia (Supiwong et al. 2013). Moreover, Pradeep et al. (2011) has chromosome metaphase spread by the modified copping method from the whole body of larvae of *O. mossambicus*×*O. niloticus* hybrid, applying colchicine treatment to the larvae. On the other hand, Mukti et al. (2016) revealed differences in arresting metaphase chromosome spreads from fin tissues of one-, two- and three-month-old Nile tilapias without using any colchicine treatment. The main aim of these studies was to obtain a large quantity of well-spread metaphase chromosome set using with a minimum possible tissue sample and in the shortest possible time.

In the current study, the metaphase index values obtained from gill, spleen, kidney, and caudal fin tissues of males are represented in Table 1. The highest metaphase index values were calculated in the Trypsin (+) Colchicine (+) treatment group of all tissues. Moreover, the scores of metaphase index found in spleen and kidney tissues were greater than those found in gill and fin tissues. Similar results were found in preparations from the tissues of females (Table 2). The results, shown in Table 3, clearly demonstrated that using colchicine and trypsin in chromosome preparations played a critical role in affecting the countability of metaphase chromosome spreads in Nile tilapia larvae. Especially, using these chemicals together has made a significant difference in the values of metaphase index.

A considerable amount of literature has discussed the effect of colchicine on maximizing the number of countable cells in metaphase while no studies have been found which examine effects of trypsin. Level of mitotic cells in sampled tissues is a decisive factor in chromosome preparations. Colchicine disrupts spindle microtubules and provides capture of the chromosome division at metaphase stage. Therefore, it improves the number of cells with metaphase chromosome. Moreover, a proper dosage of colchicine might prevent the bursting of cells and the overlapping of chromosomes during the preparation process (Cattin and Ferreira 1989; Pradeep et al. 2011). Also, colcemid which acts in a way like colchicine in terms of the disruption of spindle could be used instead of colchicine (Rieder and Palazzo, 1992; Harvey et al. 2002). Colchicine treatment to arrest cells in metaphase stage could be administered in two ways: *in vivo* or *in vitro*. In the first one, colchicine is injected into the abdominal cavity of adult individuals kept in this condition about one hour or larvae are allowed to swim for 4-6 hours (Moreira-Filho and Bertollo 1991; Blanco et al. 2012; Mukti et al. 2016). Secondly, colchicine could be applied when chopping of dissected small tissues from adults and larvae as *in vitro* colchicine treatment process (Yamazaki et al. 1981; Cattin and Ferreira 1989; Foresti et al. 1993). In the present study, it has been shown that *in vitro* colchicine treatments improved the metaphase index scores arrested in Nile tilapia tissues and larvae.

On the other hand, in the current study, *in vitro* trypsin treatment together with colchicine has been used to avoid sacrifice of adults and to prevent loss of time. Exposure to trypsin decondenses, softens and swells chromosomes. Moreover, it also allows proteolytic digestion of the chromosomal proteins resulted in more efficient Giemsa staining (O'Connor 2008; Pope et al. 2016). However, duration of trypsin exposure can affect the quality of the chromosomes (Howe et al. 2014). In the present study, metaphase chromosome spreads in a better quality were arrested in the Trypsin (+) Colchicine (+) treatment group (Figure 2). Some studies have arrested metaphase chromosome spreads without colchicine or trypsin treatments (Deng et al. 2003). In this way, some disadvantages could be avoided such as longer preparation time, chemical expenditure, and toxic effects of chemicals (Mukti et al. 2016). The present study has also shown that metaphase chromosome spreads could be obtained without colchicine or trypsin treatments (Figure 1). This could be preferable unless needed for large number of metaphase chromosome spreads and when the quality of metaphase chromosome spreads is negligible.

CONCLUSION

In conclusion, the present study was designed to determine the effects of colchicine and trypsin treatments on countable metaphase chromosome spreading according to calculation the metaphase index. The highest metaphase index values were calculated in spleen and kidney tissues while the lowest ones were detected in preparations from caudal fin. Moreover, using both colchicine and trypsin in chromosome preparations significantly affected the values of metaphase index in the tissues of adult individuals as well as in larvae. Additionally, the results indicated the possibility of arresting metaphase chromosomes without any treatment of these chemicals, especially in larvae. Further studies focusing on not just different fish species, but also different aquatic organisms including invertebrate species will need to be carried out in terms of arresting countable metaphase chromosome spreads using with colchicine and trypsin treatments.

Compliance with Ethical Standards

a) Authors' Contributions

B.E.İ. : Designed the study. Performed the laboratory work. interpreted data and drafted the manuscript.

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals

The study was conducted in Aksaray University Experimental Animal Application and Research Center (ASÜ-DEHAM) in advance with the approval number of 2021/1-3.

d) Statement of Human Rights

This study does not involve human participants.

e) Ethics Statement

This study was approved by Aksaray University Experimental Animals Application Research Center with the decision numbered 2021/1-3.

f) Supporting Institution

This study was carried out by Aksaray University Scientific Research Projects Coordination Unit within the scope of the project 2021/021

g) Project Number

Aksaray University Scientific Research Projects Coordination (2021/021)

h) Acknowledgements

I would like to thank Prof. Dr. Mustafa Öz from Aksaray University for the help he provided. In addition, the author would like to express my special thanks to Prof. Dr. Tahir Karaşahin for providing the opportunity to examine the sections under a fluorescence microscope at Aksaray University Embryo Transfer Education Application Research Center.

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TABLES

Table 1. Effects of colchicine and trypsin treatments on metaphase index from gill, spleen, kidney, and fin tissues of male Nile tilapia (*O. niloticus*)

Treatments	Gill	Spleen	Kidney	Fin
Trypsin (-) Colchicine (-)	0.031±0.008^{b}	0.126±0.035^{b}	0.109±0.022^{b}	0.010±0.002^{b}
Trypsin (-) Colchicine (+)	0.121±0.017^{a}	0.210±0.040^{ab}	0.173±0.053^{ab}	0.028±0.006^{a}
Trypsin (+) Colchicine (-)	0.084±0.018^{ab}	0.145±0.036^{b}	0.136±0.028^{ab}	0.012±0.003^{b}
Trypsin (+) Colchicine (+)	0.135±0.039^{a}	0.276±0.016^{a}	0.219±0.048^{a}	0.025±0.004^{a}

Values with different superscript letters differ significantly in each column at $p < 0.05$.

Table 2. Effects of colchicine and trypsin treatments on metaphase index from gill, spleen, kidney, and fin tissues of female Nile tilapia (*O. niloticus*)

Treatments	Gill	Spleen	Kidney	Fin
Trypsin (-) Colchicine (-)	0.027±0.002^{c}	0.120±0.019^{b}	0.099±0.003^{b}	0.012±0.001^{b}
Trypsin (-) Colchicine (+)	0.112±0.004^{ab}	0.191±0.043^{ab}	0.169±0.057^{ab}	0.020±0.003^{b}
Trypsin (+) Colchicine (-)	0.082±0.015^{b}	0.146±0.026^{ab}	0.145±0.026^{ab}	0.016±0.005^{b}
Trypsin (+) Colchicine (+)	0.143±0.026^{a}	0.224±0.036^{a}	0.210±0.040^{b}	0.029±0.003^{a}

Values with different superscript letters differ significantly in each column at $p < 0.05$.

Table 3. Effects of colchicine and trypsin treatments on metaphase index from Nile tilapia larvae (*O. niloticus*)

Uygulama	Larvae
Trypsin (-) Colchicine (-)	0.084±0.007^{b}
Trypsin (-) Colchicine (+)	0.192±0.069^{ab}
Trypsin (+) Colchicine (-)	0.155±0.032^{ab}
Trypsin (+) Colchicine (+)	0.209±0.040^{a}

Values with different superscript letters differ significantly at $p < 0.05$.

FIGURES

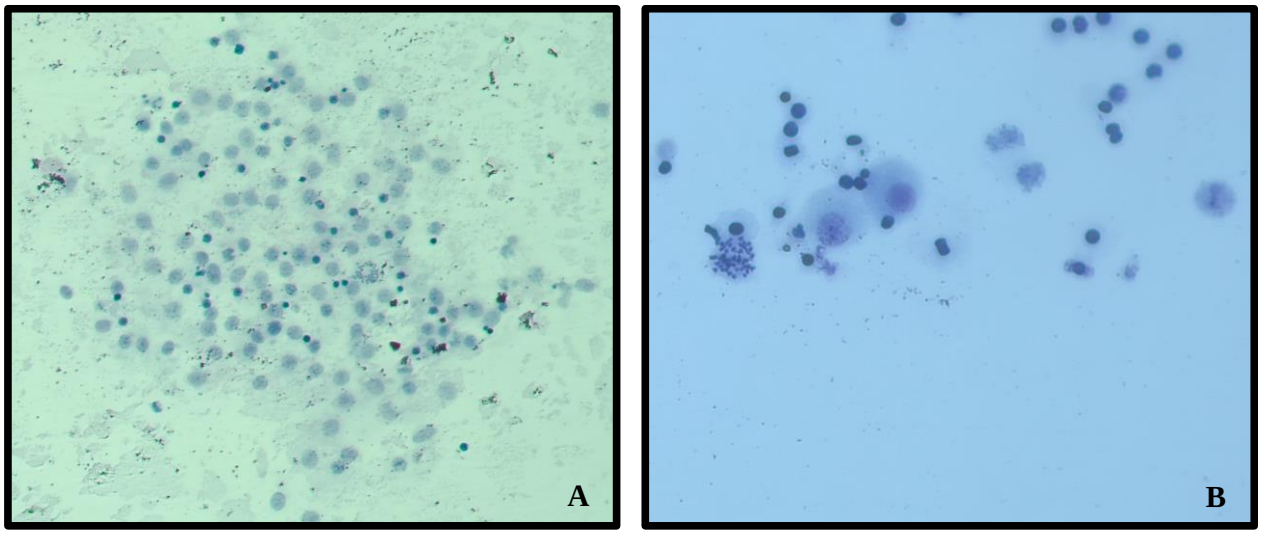

Figure 1. Metaphase chromosome spreads of Nile tilapia (*O. niloticus*) from the Trypsin (-) Colchicine (-) group (A) and the Trypsin (+) Colchicine (+) group (B).

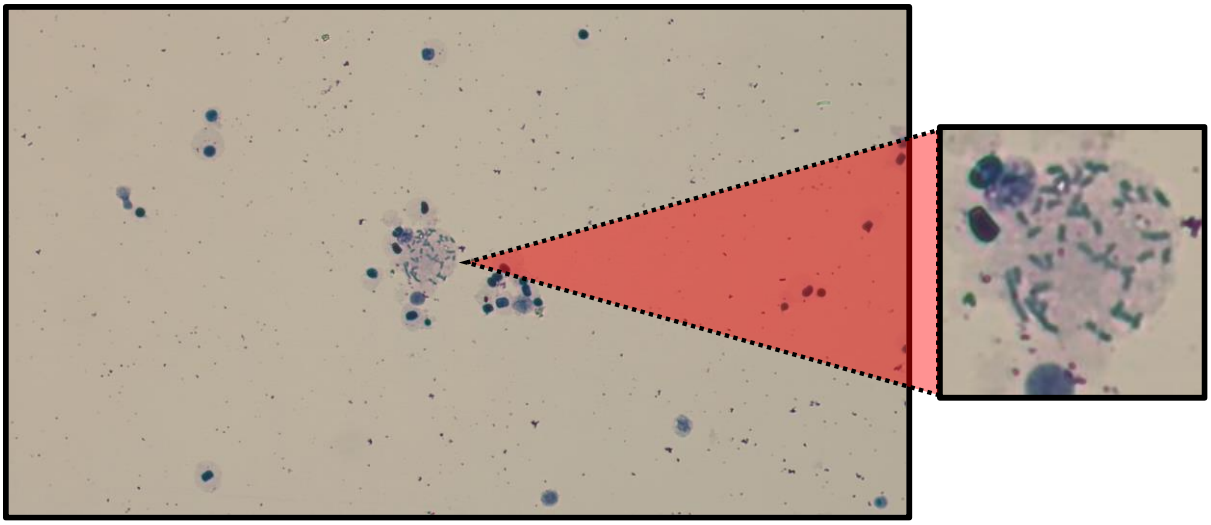

Figure 2. A metaphase chromosome spread of Nile tilapia (*O. niloticus*) from the Trypsin (+) Colchicine (+) group.