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Effect of cypermethrin insecticide on root chromosome morphometry of scallion (*Allium fistulosum*)

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Abstract. *Haq FAN, Etikawati N, Solichatun. 2023. Effect of cypermethrin insecticide on root chromosome morphometry of scallion (*Allium fistulosum*). Cell Biol Dev 7: 1-8.* Farmers in Tawangmangu, Karanganyar, Central Java, Indonesia, commonly use a pesticide known as cypermethrin insecticide whenever growing scallion (*Allium fistulosum* L.). If used at excessive levels, cypermethrin pesticide can be fatal to plants. Chromosome morphological changes are an indicator of cypermethrin insecticide's toxicity. Chromosomal morphometry, the study of chromosomal size and shape, can provide an overall picture of the quality of a plant's development. This research aimed to evaluate cypermethrin insecticide's impact on *A. fistulosum* root chromosomal morphometrics. Information gleaned from chromosomal morphometry includes chromosome shape, chromosome size, aberration type, chromosomal aberration index, relative asymmetry index, relative centromere index, and mitotic chromosome index. The concentration of cypermethrin was the sole independent variable in this study, which used a Completely Randomized Design (CRD) factorial arrangement. Long green onions were treated with varying concentrations of cypermethrin for up to four weeks: 0 mg/L, 0.05 mg/L, 0.10 mg/L, 0.15 mg/L, 0.20 mg/L, and 0.25 mg/L. The squash method was used to prepare the plant's apex roots. The root chromosomal visualization output was subjected to quantitative and qualitative descriptive analysis. The mitotic index decreased, and the chromosome aberration index increased after exposure to cypermethrin pesticides. The higher the concentration of the pesticide cypermethrin, the lower the mitotic index and the greater the chromosome aberration index. Long green onion root cells exposed to cypermethrin insecticide underwent chromosomal aberrations that could be identified qualitatively. Sticky chromosomes, chromosome bridge, chromosome agglutination, disrupted metaphase, disturbed anaphase, and hypoploid cell are examples of chromosomal aberrations.

Keywords: *Allium fistulosum*, chromosome, cypermethrin, mitosis, morphometry, scallion

INTRODUCTION

Karanganyar District, Central Java, Indonesia, is an area that acts as a center for producing horticultural crops and various other types of plants, such as food crops, ornamental plants, and medicinal plants. One flowering plant often found in agricultural areas in Tawangmangu, Karanganyar District, Central Java, is a scallion (*Allium fistulosum* L.). The *A. fistulosum*, or spring onion, is a plant usually used as a food ingredient. According to data from the Karanganyar Central Bureau of Statistics in 2018, *A. fistulosum* is one of the plants with the third largest commodity production after carrots and mushrooms in Karanganyar District.

The interviews show that most *A. fistulosum* farmers in the Tawangmangu, Karanganyar District area carry out many farming activities accompanied by applying insecticides. The application of insecticides or pest control substances is carried out to increase crop production both in quality and quantity. The type of insecticide that farmers often use is cypermethrin. Several farmers in the Tawangmangu area used different insecticide concentrations, ranging from 0.05 mg/L to 0.20 mg/L. Cypermethrin is a type of insecticide from the organophosphorus group which is known to be toxic to certain kinds of animals, such as armyworms, grasshoppers, fleas, and other pests that attack *A. fistulosum*.

According to Onuminya and Eze (2019), an increase in the concentration of cypermethrin insecticide in plants is known to increase the occurrence of chromosomal aberrations and reduce the mitotic index in the roots of garlic (*Allium sativum* L.). This condition allows for a similar effect to the cypermethrin insecticide on *A. fistulosum*. Therefore, an increase in chromosomal aberrations and a decrease in the mitotic index may indicate a cytotoxic effect of the cypermethrin insecticide given during plant growth.

The toxic properties of cypermethrin insecticide could have an unfavorable impact on plants if the application is not following the recommended dose, including on food crops such as *A. fistulosum*. The cypermethrin-type insecticide farmers use is usually applied without a standard usage concentration. Instead, it is determined based on the unit area or the number of individuals planted, so the farmers in Tawangmangu likely apply the cypermethrin insecticide excessively. Excessive exposure to insecticides can affect chromosomes in plant organs, resulting in changes in plants' morphological characters and physiological processes. Nofitahesti and Daryono (2016) stated that changes in chromosome characters in horticultural plants such as *A. fistulosum* can result in various ways, such as disrupting the cell division process.

Therefore, to determine the toxic effect of cypermethrin, it is necessary to study changes in the

chromosomal plants' characteristics exposed to cypermethrin to concentration ranges commonly used by farmers. In analyzing chromosomal characteristics, plants from the genus Allium are generally used. Plants of the Allium genus have prominent characteristics and are easy to observe. Levan (1935) states that the chromosomes in the roots of plants of the Allium genus have large sizes and are easy to count and observe. *A. fistulosum* is a cultivated plant most likely to get excessive exposure to cypermethrin insecticides, so this plant can be used to observe chromosomes in this study.

Information related to the cytotoxicity of cypermethrin insecticide on the shape and size of the chromosomes of *A. fistulosum* is challenging. Therefore, research on the effect of cypermethrin on the root chromosomes of *A. fistulosum* using chromosome morphometry measurement is needed. The observed root chromosome morphometry consisted of chromosome shape, number, size, chromosomal aberrations, and mitotic index. Therefore, this study aimed; (i) determine the effect of cypermethrin insecticide on the root chromosome morphometry; (ii) to determine the effect of different concentrations of cypermethrin insecticide on the root chromosome morphometry of *A. fistulosum*.

MATERIALS AND METHODS

Materials

This research was carried out from August to December 2020 at the Biology Laboratory of FMIPA and the Green House of the Central Laboratory of Universitas Sebelas Maret, Surakarta. Central Java, Indonesia. The main materials used in this study were *A. fistulosum*, Rizotin insecticide (100 mg/L cypermethrin content), 0.2% colchicine solution, (colchicine + ethanol + distilled water), 2% acetoorcein dye (orcein stain powder + glacial acetic acid + distilled water), glacial acetic acid, 1 M hydrochloric acid, distilled water, and glycerin.

Research design

This study used a Completely Randomized Design (CRD) with 1 (one) factor at the planting stage of *A. fistulosum*, namely the concentration of cypermethrin insecticide. Cypermethrin insecticide was made into six different concentrations, namely 0 mg/L (control), 0.05 mg/L, 0.10 mg/L, 0.15 mg/L, 0.20 mg/L, and 0.25 mg/L. Each treatment was made of five replications.

Procedure

Preparation of test plants (planting)

Each *A. fistulosum* seedling was planted in a polybag with a soil-compost mixture of 1:1 and placed in the Green House of Universitas Sebelas Maret. The seeds used the following criteria: (i) Seedlings were obtained from seeds that had been sown for ±4 weeks. (ii) Seedlings have no insecticide history application during seeding. (iii) Seedlings are planted in a planting medium in the form of soil mixed with compost in a ratio of 1:1. (iv) The *A. fistulosum* plant media is watered regularly every two days until the plants are ± eight weeks old or ready to be harvested.

Insecticide treatment

Insecticide treatment used six different concentrations, namely 0 mg/L (the control), 0.05 mg/L, 0.10 mg/L, 0.15 mg/L, 0.20 mg/L, and 0.25 mg/L. Insecticide formulations were sprayed on *A. fistulosum* from the 5th to the 8th week of age. Cypermethrin insecticide was applied on all parts of the plant above the ground using a 1 mL spray bottle for each test plant. The intensity of one time per week and in the afternoon.

Chemical manufacture

Colchicine solution 0.2%. Colchicine 0.2 g dissolved in 5 mL ethanol, then stirred until dissolved. After dissolving, 95 mL of distilled water was added and stirred until thoroughly mixed. The solution is stored in glass bottles.

Glacial acetic acid 45%. The 45% glacial acetic acid was prepared by mixing 45 mL of pure glacial acetic acid with 55 mL of distilled water, then stirring until smooth. The solution was stored in a dark bottle and at room temperature.

Acetoorcein 2% dye. Aceto orcein 2% is prepared by heating 45 mL of glacial acetic acid to almost boiling (90-100°C). After that, it is mixed with 2 grams of orcein powder and boiled for 10 minutes while continuing to stir. The solution was left to cool to room temperature, and then 55 mL of distilled water was added and stirred again until evenly distributed. Finally, the acetoorcein dye solution was filtered and stored in a dark bottle at room temperature.

1M HCl. Hydrochloric acid (HCl) 1 M is prepared from diluted 2 M HCl. Dilution was performed by mixing 50 mL of 2 M HCl with 50 mL of distilled water (1:1 ratio). The solution was stored in a dark bottle and at room temperature.

Chromosome preparation

Root chromosome preparations were made using the semi-permanent squash method. In the pre-treatment process, the root tips were cut 0.5-1.0 cm, soaked with 0.2% colchicine in a flacon bottle, and incubated in the refrigerator at 4°C for 4 hours. The root samples were then washed with distilled water three times. Following the fixation process, the root samples were soaked in 45% glacial acetic acid and incubated in the refrigerator at 4°C for 15 minutes. The root samples were then washed with distilled water three times. After hydrolysis, the root samples were soaked in 1M HCl and incubated in an oven at 60°C for 5 minutes. The root samples were then washed with distilled water three times. The next stage was the coloring process; the root samples were soaked in 2% acetoorcein dye solution for 1 hour and stored at room temperature (±25°C). After that, the root samples were taken and placed in glass objects. The root sample was dripped with glycerin and covered with a glass cover. The glass preparation is placed on a flat plane, then pressed (squash) or tapped gently until the sample is evenly crushed. The glycerin oozing out of the edges of the coverslip was cleaned, then sealed with clear nail polish.

Chromosome observations

Observations of root chromosome morphometry were performed using a light microscope. The ocular lens magnification was 100x, and the objective lens magnification was 10x (total magnification 1000x). Moreover, to improve the resolving power, immersion oil is used on the surface of the preparation. Preparations that can be observed are visualized with a digital microscope and the NIS-Elements F3.0 program to be documented in photographs. Sahin and Koca's (2018) observations of the visualization results of root chromosomes were carried out in 500 cells for each treatment sample. Photos of visualization results are observed and determined if chromosomal aberrations occur. Chromosomal aberrations can be in the form of laggard chromosomes, c-mitotic, chromosome bridges, and others.

Chromosome measurement

The results of the chromosome visualization were processed again with ImageJ Fiji version 1.51h to calculate the number and size of each chromosome arm. A total of five cells that were still actively dividing at the prometaphase stage from each treatment sample were selected to measure chromosomal characters. First, the number of chromosomes was counted manually per unit cell, then the size of the chromosomes was measured in micrometers (µm). Chromosome sizes include long arms, short arms, and the entire chromosome body.

Calculation of chromosome morphometry data

The results of the chromosome size calculation were then processed again to determine the quantitative data of chromosome morphometry. The result is in the form of relative centromere index (Ci%), relative asymmetry index (AsI%), total aberration index (TA%), and mitotic index (IM%). The calculation formula used:

Relative centromere index (Ci%)

$$Ci\% = \frac{short\ sleeve\ size}{total\ sleeve\ size} \times 100$$

Relative asymmetry index (AsI%)

$$AsI\% = \frac{long\ sleeve\ size}{total\ sleeve\ size} \times 100$$

Total aberration index (TA%)

$$TA = \frac{\text{the number of cells experiencing aberration}}{\text{the total number of dividing cells}} \times 100$$

Mitotic index (IM%)

$$IM = \frac{\text{the number of cells in the mitotic phase}}{\text{the total number of cells observed}} \times 100$$

Data analysis

Descriptive qualitative and quantitative are used to analyze data in this research. The observed data of chromosomal aberrations are described qualitatively. The morphometric data was then processed by calculating the number of chromosomes for each cell, the size, and the shape of chromosomes. The chromosomal morphometry data were analyzed using the SPSS 16.0 program through the DMRT test of one-way Analysis of Variance (OneWay ANOVA) with a 95% confidence level to assess the effect of the treatment factors.

RESULTS AND DISCUSSION

Chromosome morphology

Qualitative and quantitative characterization of *A. fistulosum* root chromosomes was conducted to detect changes in chromosome characters after being treated with insecticides during its growth period. Qualitatively, the type of chromosomal aberration can be determined when the cell is actively dividing. Setyawan and Sutikno (2000) stated that in Allium plants is more apparent at the prometaphase stage. This is because the location of the chromosomes would be more spread out, and the shape of the centromere indentation is more apparent at the prometaphase stage. In observing the sample without treatment (the control), some cells are still active in the prometaphase division phase. These cells are considered representative cells to show normal chromosomal characteristics when actively dividing cells without the effect of the treatment given (Figure 1).

In the process of dividing at the prometaphase stage, visualizing cells is then made. A karyotype map and details of the morphological characters of the chromosomes are carried out. Arsal (2018) stated karyotype map shows how many chromosomes are present in a cell with some details of the structure of the chromosomes and the number of chromosomes. For example, *a. fistulosum* is known to have a diploid number of chromosomes (2n) with eight pairs of homologous chromosomes. Suminah et al. (2002) stated that most cultivated onion plants are diploid with a primary chromosome number of eight (x=8), so 2n=16. The preparation and results of the karyotype map from normal representative cells without treatment (the control) in this study are shown in Figures 2 and 3.

Moreover, to determine the presence of chromosomal abnormalities in a cell, it is necessary to have a quantitative analysis by calculating the relative centromere index (Ci%) and relative asymmetry index (AsI%). According to Qurniawan et al. (2012), the calculation of the relative centromere index (Ci%) and relative asymmetry index (AsI%) requires that the chromosome size is known by measuring the length of the chromosome arms. Therefore, chromosome measurements from each treatment sample yielded relative centromere index (Ci%) and relative asymmetry index (AsI%) values, presented in Table 1.

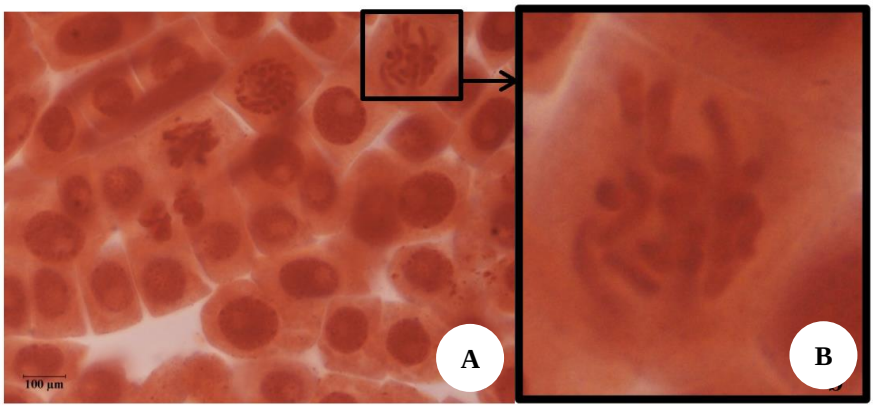

Figure 1. Results of observing the root cell chromosomes of *Allium fistulosum.* Note: A. magnification 1000x, B. Prometaphase stage of cell division

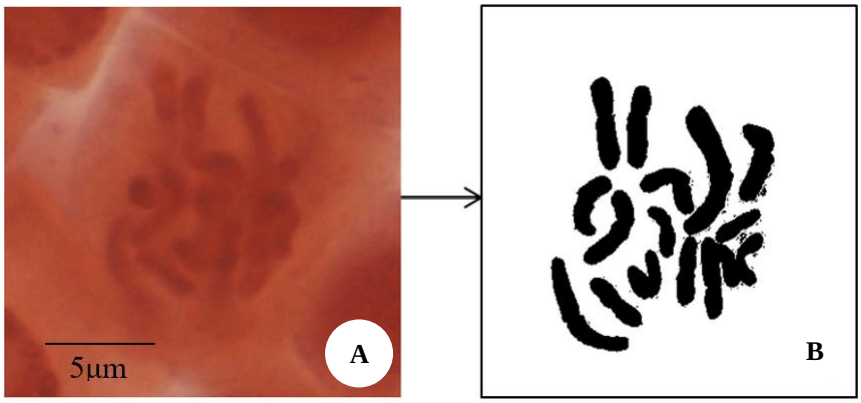

Figure 2. The results of observing the root cell chromosomes of *Allium fistulosum* at the prometaphase stage. Note: A. Prometaphase root cells, B. Chromosome schematic drawing

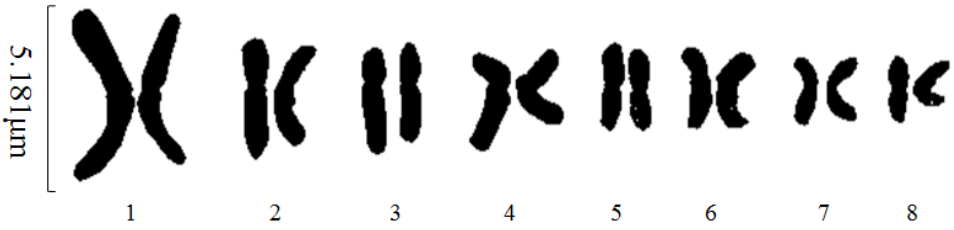

Figure 3. Karyotype map of *Allium fistulosum*

Table 1. The effect of different concentrations of cypermethrin insecticide on the quantitative character of the root chromosomes of *Allium fistulosum*

Cypermethrin concentration	$\bar{x}_p$	Ci%	AsI%
0 mg/L	3.59 µm	39.68	60.31
0.05 mg/L	5.84 µm	38.56	61.43
0.10 mg/L	6.01 µm	37.88	62.11
0.15 mg/L	5.10 µm	41.26	58.73
0.20 mg/L	4.43 µm	41.30	58.69
0.25 mg/L	5.48 µm	39.45	60.54

Note: The average value of total chromosome arm length (p), relative centromere index. (Ci%), and relative asymmetry index (AsI%)

Based on Table 1, the concentration of cypermethrin insecticide was assessed as not affecting the number and shape of the root chromosomes of *A. fistulosum.* The result shows that each difference in the concentration of cypermethrin gave normal levels. That same result as in the samples without cypermethrin insecticide treatment (the control). The six treatments with different concentrations had the same number of chromosomes, namely 2n = 16, and the chromosome pairs were dominated by metacentric chromosomes (Ci% = 37.50-50.00). The relative asymmetry index (AsI%) results indicate that the chromosomes are asymmetric in shape because there are also many sub-metacentric and telocentric chromosomes.

Setyawan and Sutikno (2000) suggested that a relative asymmetry index with a value of >50% indicated that symmetrical (metacentric) chromosomes were not dominant. In each sample, all chromosomes can be found in metacentric, sub-metacentric, and telocentric.

Mitotic index and chromosomal aberration index

The root mitotic index of *A. fistulosum* was influenced by the administration of cypermethrin insecticide and concentration changes, as determined by statistical analysis. The mitotic index value is the percentage of cells that undergo division in the observed samples. The mitotic index was often higher in the control group than in the cypermethrin-treated group. Likewise, cypermethrin differences are the higher the concentration, the lower the average mitotic index. The sample with the highest concentration of cypermethrin insecticide (0.25 mg/L) had the lowest mitotic index value (3.24%), whereas the sample without treatment (the control) had the highest mitotic index value (14.8%) (Figure 4).

The existence of cytotoxic characteristics generated by cypermethrin insecticide on *A. fistulosum* is evidenced by a decrease in the mitotic index. That is directly proportionate to an increase in the concentration of cypermethrin insecticide. It is consistent with the findings of Yakeen and Adeboye (2013), who noted that cypermethrin is a commonly used pyrethroid class insecticide for the upkeep of cultivated plants. Multiple studies have shown cypermethrin insecticides to cause significant chromosomal aberrations and mitotic division inhibition. Sahin and Koca (2018) added that as the mitotic index value drops, followed by mitotic activity drops along with it, which signals that DNA synthesis is being suppressed in the cell. There is speculation that the pesticide cypermethrin's cytotoxic characteristics could slow down DNA replication.

This study shows that cypermethrin pesticide is thought to cause chromosomal abnormalities and a drop in the mitotic index in the roots of *A. fistulosum.* The chromosomal aberration index measures the frequency of chromosomal abnormalities in actively dividing cells. Therefore, *a. fistulosum* roots were analyzed quantitatively to determine the chromosomal aberration index, and the findings are depicted in Figure 5.

The root aberration index of *A. fistulosum* was found to be affected by both the application of cypermethrin insecticide and concentration variations. Cypermethrin insecticide was found to have cytotoxic effects on actively proliferating *A. fistulosum* root cells, as evidenced by increased chromosomal aberration index value at higher dosages. The degree of chromosomal abnormalities was lower in the 0.25 mg/L cypermethrin insecticide treatment than in the 0.20 mg/L treatment. That treatment shows cells in the 0.25 mg/L treatment still in their cell cycle's interphase and prophase stages. Due to the slowed rate of cell division, abnormalities cannot be detected. According to studies by Chandraker et al. (2014), measuring the extent to which interphase cell division is dominant can indicate mitotic cycle suppression caused by hazardous chemicals.

Chromosomal aberrations

Based on qualitative observations of chromosomal aberrations, multiple types of aberrations were observed in *A. fistulosum* root cells from all samples, ranging from untreated samples to those treated with 0.25 mg/L of cypermethrin insecticide. Chromosomal aberrations are a qualitative description of cytological mutations in cells. Sahin and Koca's (2018) research indicates that a decrease in the mitotic index and the presence of chromosomal aberrations in the form of laggard chromosomes, micronuclei, and sticky chromosomes indicate the presence of cytotoxic properties of foreign substances. That also indicates the affected meristematic cells during division.

According to Rao et al. (2005), high quantities of cypermethrin cause chromosomal aberrations in metaphase and chromosome bridge creation in anaphase. In this research, sticky chromosomes (Figure 6); chromosome bridge (Figure 7); chromosome agglutination (Figure 8); disrupted metaphase (Figure 9), and disturbed anaphase were identified as chromosomal aberrations (Figure 10). The following explains the sticky chromosome, chromosome bridge, chromosome agglutination, disrupted metaphase, and disturbed anaphase.

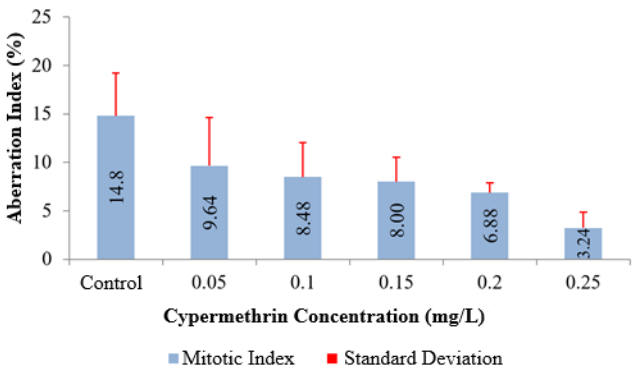

Figure 4. The relationship between the concentration of cypermethrin insecticide and the root mitotic index of *Allium fistulosum*

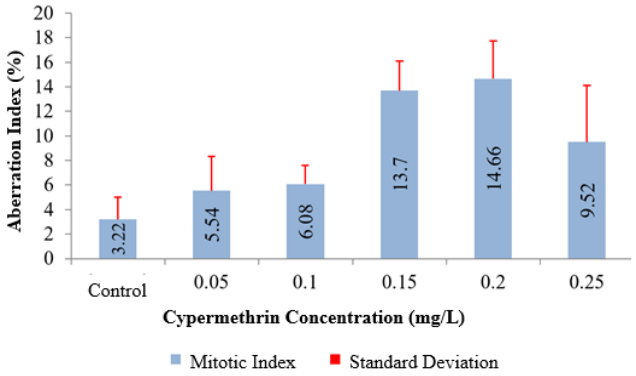

Figure 5. The relationship between the concentration of cypermethrin insecticide and the chromosomal aberration index of the roots of *Allium fistulosum*

A sticky chromosome is a type of chromosomal aberration characterized by the attachment of several chromosome numbers to one another, hence limiting mitotic division. According to Rosculete et al. (2019), the sticky chromosome is the most prevalent form of chromosomal aberration observed at the terminals of Allium roots. The aberration of sticky chromosomes represents a significant toxicity effect and can result in cell death. The comparatively high toxicity of pyrethroid group chemicals on Allium plant roots causes irreversible physical damage to the chromosomal proteins in the arrangement of the cell's chromosomes (Yakeen and Adeboye 2013). Figure 6 depicts the results of observations of sticky chromosomal aberration.

(ii) Chromosome bridge or chromosomal bridge is an anaphase-stage chromosomal aberration characterized by the presence of chromosome arms between the two planes of division. Figure 7 depicts the findings of observations of chromosomal bridge abnormalities. According to Imaniar and Pharmawati (2014), chromosome bridges can arise due to inversion (re-insertion) during cross-over. That inversion causes the inverted portion to generate a centromere, resembling a bridge to another chromosome. In addition, according to Yakeen and Adeboye (2013), a significant number of chromosome bridges indicate cytotoxic chemical damage.

(iii) Chromosome agglutination or chromosome stacking, specifically chromosomal aberrations in which the chromosomes in the nucleus overlap in one field while the other is vacant. Figure 8 depicts the findings of observations of chromosomal agglutination abnormalities. According to Imaniar and Pharmawati (2014), mutagens in the form of excessive pesticide chemicals cause the fragmentation of chromosomes, which subsequently accumulate in one place.

(iv) In the abnormal metaphase and anaphase phases, the disturbances form as a chromosomal abnormality. Disturbed metaphase is a condition in which the metaphase plate cannot form correctly due to faulty cell structure and location, whereas disturbed anaphase is a condition in which the spindle and microtubule orientation is disturbed. According to El-Araby et al. (2020), the primary cause of decreasing percentage of cell division is the disruption of metaphase and anaphase. As a result of the toxic properties of foreign nanoparticle material inserted between microtubules and affecting the mitotic division process in cells. Furthermore, disturbed metaphase and disturbed anaphase are cited as side effects. Figures 9 and 10 depict the result of observations on chromosomal abnormalities in disturbed metaphase and anaphase.

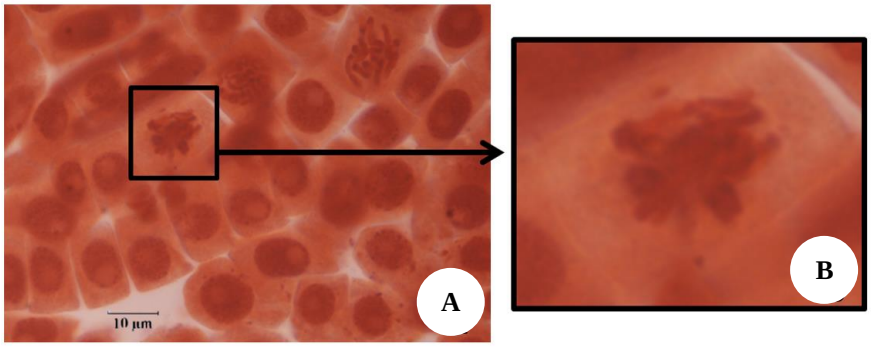

Figure 6. Results of observations of chromosomal aberrations in the root cells of *Allium fistulosum.* Note: A. magnification 1000x, B. sticky chromosome

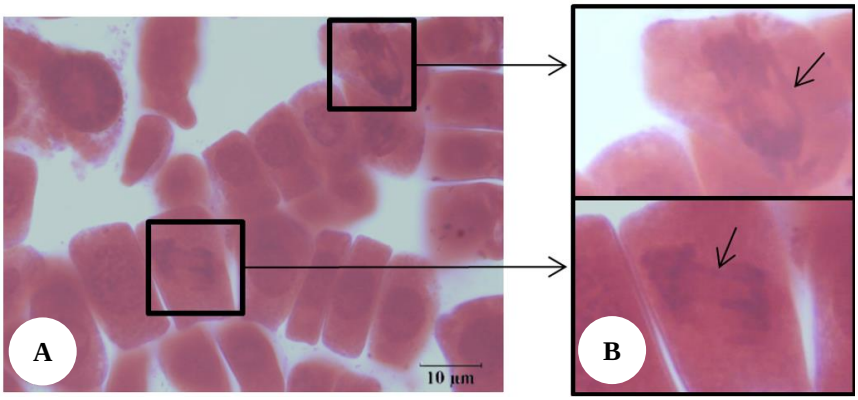

Figure 7. Results of observations of chromosomal aberrations in the root cells of *Allium fistulosum.* Note: A. magnification 1000x, B. chromosome bridge

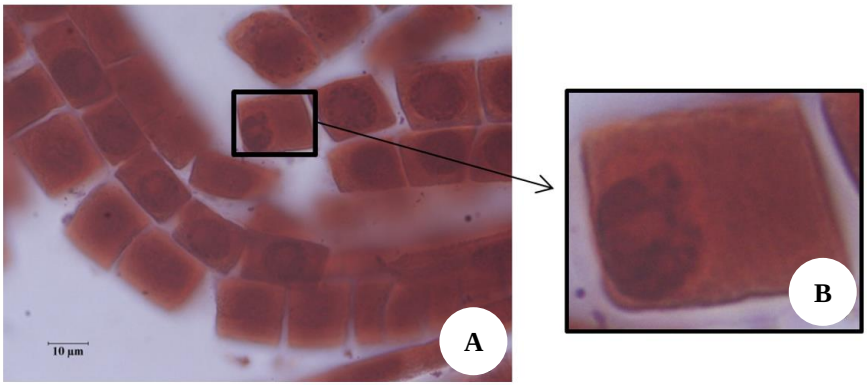

Figure 8. Results of observations of chromosomal aberrations in the root cells of *Allium fistulosum*. Note: A. magnification 1000x, B. chromosome agglutination

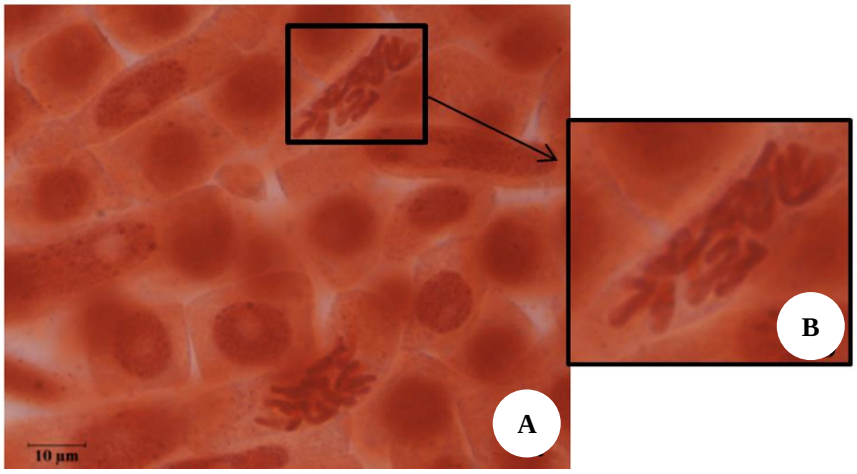

Figure 9. Results of observations of chromosomal aberrations in the root cells of *Allium fistulosum*. Note: A. magnification 1000x, B. disturbed metaphase

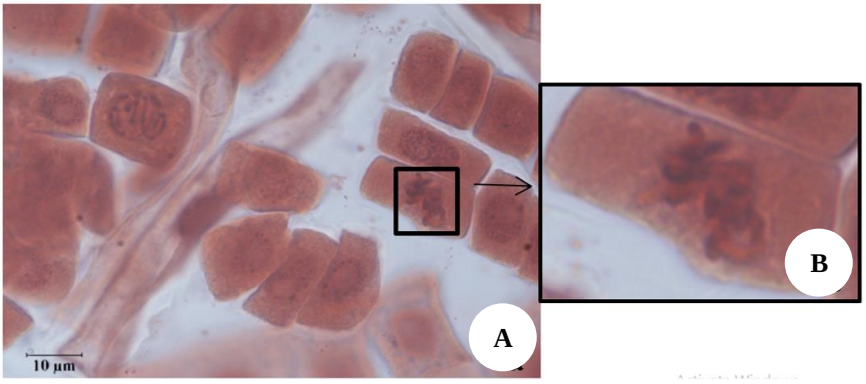

Figure 10. Results of observations of chromosomal aberrations in the root cells of *Allium fistulosum*. Note: A. magnification 1000x, B. disturbed anaphase

The presence of chromosomal abnormalities in the root cells of *A. fistulosum* suggests that cypermethrin insecticide has a direct influence on cell division and will have a negative effect on the growth of *A. fistulosum*. When used excessively, cypermethrin insecticides' toxicity can potentially affect cultivated plants' growth. The use of cypermethrin insecticides on *A. fistulosum* necessitates more specific instructions regarding the ideal concentration level for particular plant units to preserve proper plant growth.

Based on the provided results and discussion, the following conclusions are as follows; (i) The insecticide cypermethrin influences the root chromosomal morphometry of *A. fistulosum*. In response to the chromosomal index and chromosomal aberrations, the mitotic index value decreased, and the chromosomal aberration index value increased. Sticky chromosomes, chromosome bridge, chromosome agglutination, disrupted metaphase, and disturbed anaphase is examples of chromosomal abnormalities. (ii) Different cypermethrin insecticides dosage influence the chromosomal morphometry of *A. fistulosum* roots. The higher the concentration of the pesticide cypermethrin, the lower the mitotic index and the higher the chromosome aberration index.

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