Cytological and phytochemical studies on *Mesembryanthemum nodiflorum* L.

Aya M. Abdel gawad[1] , Sahar A. Tawab[1], Shaimaa S. Sobieh[1], Dina M. Fahmy[2].

1- Botany department, Faculty of women for Arts, Science and Education, Ain Shams University.

2- Desert Research Center

Abstract

Mesembryanthemum nodiflorum L. (Aizoaceae) has long been used as food and in traditional medicine. This study was intended to explore the active groups within *M. nodiflorum* ethanolic extract and to study the cytological effects of *M. nodiflorum* water extract on *Allium cepa* L. root tips. Phytochemical analysis of *M. nodiflorum* ethanolic extract indicated the presence of alkaloids and triterpenes. Tannins, flavonoids and saponins did not found in *M. nodiflorum* alcoholic extract. Mitotic indices and distribution of cells in mitotic phases of *A. cepa* root tips were clearly changed after treatment with three different concentration of *M. nodiflorum* water extract (0.1, 1 and 3%) for 3h, 6h, and 12h. The maximum value of mitotic index 2.68% was observed after treatment with 3% for 6h, while the minimum value 1.35% was scored after 12h exposure to the same concentration. Different types of chromosomal aberrations were noticed. The present study revealed that *M. nodiflorum* water extract has a potent inhibitory effect on the mitotic activity of *A. cepa* root tip cells.

Keywords: *M. nodiflorum*, *A. cepa*, Mitotic index, Phytochemical screening

1. Introduction

Aizoaceae "weedy mesembs" is considered as one of the most widely distributed plant families in Africa. Two subfamilies are recognized: the Mesembryanthemoideae and the Ruschioideae **(Klak *et al.*, 2004; Smith *et al.*, 1998)**. The subfamily of Ruschioideae is the largest and contains 101 genera and 1563 species and representing about 85% of the *Aizoaceae* **(Klak *et al.*, 2003)**. While the subfamily of Mesembryanthemoideae, has approximately 102 species of the genus *Mesembryanthemum* **(Klak *et al.*, 2007)**, and is distributed in Mediterranean region, Atlantic island, Saudi Arabia, South Africa, South Australia and California **(Boulos, 1999)**.

According to **Boulos (1999)**, 6 genera and 10 species of the family Aizoaceae are listed in Egypt. In Egyptian flora, three *Mesembryanthemum* species were recorded (*Mesembryanthemum crystallinum* L., *Mesembryanthemum nodiflorum* L. and *Mesembryanthemum forsskaolii* Hochst. ex Bioss) **(Soliman *et al.*, 2014).**

Several reports have investigated many biological activities of *M.crystallinum* (common ice plant) such as antioxidant, antimicrobial and antiviral activity **(Falleh *et al.*, 2011).** *M. forsskaolii* (samh plant) seeds contain high protein content so it is used as a replacement for wheat for baking **(Mustafa *et al.*, 1995).** Moreover, *M. nodiflorum* is an important medicinal

material that is used in traditional Tunisian medicines for treatments of ocular infection **(Chaieb and Boukhris, 1998; Soliman *et al.*, 2017).**

Phytochemical studies reveal that plants in this family contain tannins, triterpenes, alkaloids and saponins as reported by **Doudach *et al*. (2013).**

Corresponding author: ayamohamed9045@yahoo.com

The present study aims to explore the active groups within the *M. nodiflorum* ethanolic extract and to study the cytological effects of water extract on the mitotic division of *Allium cepa* L. root tip cells. The *Allium* assay is a fast and sensitive assay used extensively for detection of environmental genotoxins and mutagens. **Leme and Marine- Morales, (2009)** reported that the *A. cepa* test is used in environmental monitoring of chemicals and provides a high degree of correlation with the gentoxicity assays of both human and mammalian.

2. Materials and methods

2.1. Plant materials

A) Plant Samples of *Mesembryanthemum nodiflorum* L. Family: Aizoaceae were collected by Aya M. Abdel Gawad, identifyed and authenticated in Herbarium of Botany department, Faculty of Science, Ain Shams University. The collection were in three successive years between March and April (2014-2015-2016) from Petrified Forest Protected Area, Egypt.
B) Bulbs of *Allium cepa* L. (2n=16) Giza 6 mohassan were obtained from Agricultural Research Centre.

2.2. Extract preparation

Fresh samples of plants (5gm) were weighted and oven- dried at 40°C. The dried samples were extracted with distilled water at 55-60°C for cytological study several times, filtered, concentrated and allowed to dry till constant weight was obtained. While, the phytochemical study was done by using 70% ethanol extract.

2.3. Phytochemical screening

It includes: tests for tannins, triterpenes, flavonoids, alkaloids and saponins.

Test for tannins: 2-3 drops of $FeCl_3$ (5%) and 2 ml distilled water were added to 2 ml plant alcoholic extract, if green precipitate was formed then tannins is present **(Balbaa, 1986; Ramamurthy and Sathiyadevi, 2017)**.

Test for triterpenes: one ml of acetic anhydride was added to 2ml plant alcoholic extract, followed by the addition of sulphuric acid down the wall of the test tubes; a deep red coloration produced, indicating the presence triterpenes (**Lieberman1885; Burchard, 1890)**.

Test for Flavonoids: 1 ml of 10% lead acetate was added to 1 ml of extract, a yellow color means the presence of flavonoids **(Geissman, 1962).**

Test for alkaloids: a few drops of Hager's reagent were added to 2 ml of extract, yellow precipitate was produced, indicating the presence of alkaloids. **(Harborne, 1973)**

Test for saponins: to the powder 10 ml of distilled water was shaken, heated; froth appeared indicating the presence of saponins **(Wall et al., 1954 and Balbaa 1986)**

2.4. Cytological analysis

Allium cepa L. bulbs were used in this analysis, according to **Levan, (1938)** and **Fiskesjo, (1988)**. The bulbs were soaked in distilled water for 48 h at room temperature until the roots reached 3—4 cm in length. The water was replaced by the different concentrations of *M. nodiflorum* water extract 0.1, 1 and 3% for 3, 6 and 12 hours for each concentration. Distilled water has been used for the control experiments. The roots were cut and fixed in Carnoy's solution for 48 h, hydrolyzed with 1N HCl at 60° C for 5 min., washed with distilled water and stained using leucobasic fuchsin stain for 1h in the dark. The meristematic region were carefully squashed in 45% acetic acid then examined at 400× magnification using an Olympus Microscope (BX50, Olympus Company, Tokyo,Japan). Replicates of five bulbs were analyzed for each treatment and control. Mitotic index was calculated and defined as the number of dividing cells per 1000 cells. Phase index and percentage of mitotic abnormalities were scored. Cells with the most representative types of aberration were photographed.

3. Results

3.1. Phytochemical screening

The preliminary phytochemical screening on *M. nodiflorum*; revealed the presence of alkaloids and triterpenes. No saponins, tannins or flavonoids were detected in *M. nodiflorum* as shown in table 1.

Table 1. The preliminary phytochemical screening of *M.nodiflorum* ethanolic extract.

Constituents	**Ethanolic extract of *M.nodiflorum***
Tannins	-
Flavonoids	-
Triterpenes	+
Saponins	-
Alkaloids	+

3.2. Cytological analysis

A significant universal depression in the mitotic index was observed in *Allium cepa* roots treated with *M. nodiflorum* water extract as compared with its control (Table 2 and figure 1). The maximum value of mitotic index 2.68% was observed after treatment with 3% for 6h, while the minimum value of the mitotic 1.35% was scored after 12h exposure to the same concentration. The accumulation of cells at prophase stage was noticed as can be seen in table 2. The highest ratio of prophase frequency was 43.53% that took place after 12h exposure to 3%. While the minimum ratio of prophase was 30.84% which caused by treatment with 1% for 12h. (Table 2)

Metaphase stage scored the highest percentage after treating *Allium cepa* root tips with 3% of water extract for 12h reaching 35.29%. Treatment with 0.1% was able to induce the least percentage of metaphase reaching 25.49% after 3h exposure.

The maximum ratio of anaphase stage was 25.30% that took place after 12h exposure to 1% water extract. The minimum ratio of anaphase stage was 10.42% which caused by the treatment with 0.1% for 6h exposure.

Telophase stage recorded the maximum frequency following treatment with 0.1% for 3h reaching 24.51%, while 10.59% was the minimum frequency of telophase after exposure to 3% for 12h.

Treatment *Allium cepa* roots with *M. nodiflorum* water extract resulted in a significant increase in the percentage of total abnormalities which exceeded its counterpart control, this increasment occurred after all concentrations. The concentration of 1% of *M. nodiflorum* water extract induced the highest percentage of mitotic abnormalities in *Allium cepa* root cells reaching 21.23% after exposing root tips for 6h. The least percentage of total mitotic abnormalities was 8.82% after 3h exposure to 0.1% of *M. nodiflorum* water extract. (Table 2 and figure 2)

Different chromosomal aberrations were observed such as irregular prophase, stickiness and disturbance. Stickiness was the most common abnormality which appeared after the application 3% for 6h reaching 84.58%. The minimum percentage of stickiness was 46.84% which was scored after 3h exposure to 1% of *M. nodiflorum* water extract. Representative aberrations are illustrated in table 3 and figure 3.

Table 2. Mitotic and phase index and percentage of mitotic abnormalities of *Allium cepa* L. root tip cells treated with water extract of *M.nodiflorum*.

Treatment	Mitotic index ± SD	Mitotic abnormalities	Prophase	Metaphase	Anaphase	Telophase
Control						
3 hrs	2.72 ± 1.10	0.36 ± 0.01	24.75 ± 0.42	24.63 ± 0.81	21.38 ± 0.52	29.24 ± 0.81
6 hrs	4.22 ± 0.35	0.21 ± 0.14	25.54 ± 0.56	24.54 ± 0.30	20.40 ± 0.34	29.57 ± 0.64
12 hrs	3.97 ± 0.50	0.10 ± 0.09	25.26 ± 0.61	25.59 ± 0.12	22.28 ± 0.26	26.87 ± 0.38
0.1%						
3 hrs	2.02 ± 0.11*	8.82 ± 0.79*	36.27 ± 0.20*	25.49 ± 0.69*	13.73 ± 0.31*	24.51 ± 0.99*
6 hrs	1.87 ± 0.80**	19.79 ± 0.25**	42.71 ± 0.44*	31.25 ± 0.77*	10.42 ± 0.53*	15.62 ± 0.83*
12 hrs	1.82 ± 0.62*	14.85 ± 0.49*	37.62 ± 1.35*	29.71 ± 0.46*	18.81 ± 0.90*	13.86 ± 0.42*
1%						
3 hrs	2.44 ± 0.21**	18.70 ± 0.37*	33.09 ± 0.75*	28.78 ± 0.80*	17.27 ± 0.20*	20.86 ± 0.39**
6 hrs	1.96 ± 0.14*	21.23 ± 0.13*	35.40 ± 0.47*	29.20 ± 0.58**	16.81 ± 0.17*	18.58 ± 0.46*
12 hrs	2.01 ± 0.32*	18.69 ± 0.80*	30.84 ± 0.87*	28.91 ± 0.16*	25.30 ± 0.51*	14.95 ± 0.39*
3%						
3 hrs	2.43 ± 0.40*	14.20 ± 0.19*	35.50 ± 0.52*	27.81 ± 0.62**	21.31 ± 0.90*	15.38 ± 0.31*
6 hrs	2.68 ± 0.32*	13.57 ± 2.06*	32.86 ± 0.34*	27.86 ± 0.66*	24.28 ± 1.20*	15.00 ± 0.35**
12 hrs	1.35 ± 0.24**	11.76 ± 0.96*	43.53 ± 0.38*	35.29 ± 0.79*	10.59 ± 0.82*	10.59 ± 0.64*

* Significant compared to its control at 0.05 level

** Significant compared to its control at 0.001 level

Table 3. Types of mitotic abnormalities and their percentage induced in *Allium cepa* L. root tip cells treated with water extract of *M.nodiflorum*.

Treatment	Irregular prophase	Stickiness	Spindle disturbance
0.1%			
3 hrs	9.17	81.24	9.59
6 hrs	28.72	59.60	11.63
12 hrs	23.44	71.07	5.49
1%			
3 hrs	40.95	46.84	12.21
6 hrs	45.29	51.28	3.43
12 hrs	43.85	47.88	8.27
3%			
3 hrs	15.86	71.51	12.63
6 hrs	11.00	84.58	4.42
12 hrs	43.20	56.80	00.00

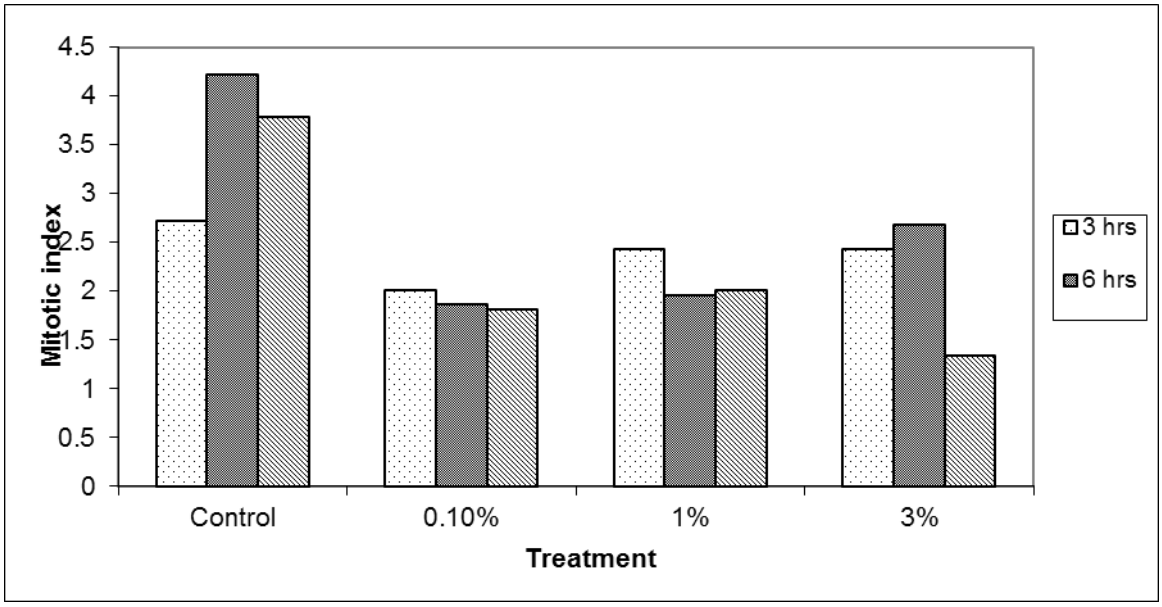


Figure 1. Mitotic index of *Allium cepa* L. root tip cells treated with *M.nodiflorum* water extract

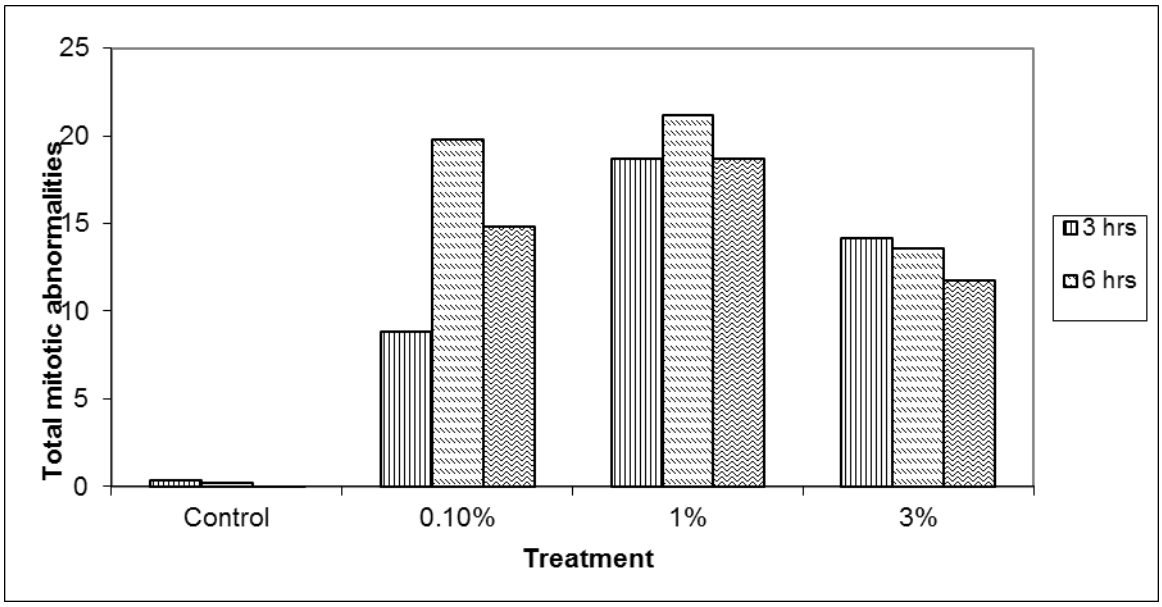


Figure 2. Total mitotic abnormalities of *Allium cepa* L. root tip cells treated with *M.nodiflorum* water extract

4. Discussion

4.1. Phytochemical screening

These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc. **(Negi *et al.*, 2011)**

From phytochemical screening, the ethanolic extract of *M. nodiflorum* was found to possess triterpenes and alkaloids. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties **(Rabi and Bishayee, 2009; Wagner and Elmadfa, 2003).**

Alkaloids have been reported as one of the important groups of phytoconstituents obtained from natural sources. It plays an important role in the ecology of organisms which synthesize them. Alkaloids play an important role in the defense mechanism against pathogens. **(Patel *et al.*, 2012)**

4.2. Cytological analysis

Cell division is one of the most important phenomenons, which controls the growth of an organism. Moreover, the behavior of chromosomes during cell division is one of the unique features of cell division. In this study, the mitotic index values showed reduction in all the treated root tip cells than the values calculated for control. This suggests that the water extract of *M. nodiflorum* inhibit the mitotic activities of *Allium cepa* root tip cells. Reduction in mitotic index was previously reported after the use of several plant extracts as: *Boerhaavia*

diffusa and *vernonia amygdalina*, **(Ene-Obong and Amadi, 1987)**; *Glycyrrhiza glabara*, **(Adam and El-Sedawy, 1988)**; *Cymbogon proximus* **(Adam and Farah, 1989)**; *Ruta graveolens,* **(El-Nahas *et al.,* 1992)**; *Artimisia herba alba,* **(Shehata *et al.,* 1999)** and *Rubus sancatus,* **(Sobieh *et al*.,2014)**

The analysis of the frequency of particular stages of mitosis points to an universal accumulation of prophases after all treatments. This accumulation was mainly on the expense of metaphases, anaphase and telophase. The prophase accumulation was previously noticed in treated *A. cepa* meristematic cells by **Sobieh *et al.,* (2014). Shehab (1980),** attributed the accumulation of *A. cepa* cells in prophase to a disturbance or breakdown of spindle apparatus. Moreover, **Odeigah *et al.,* (1997)** reported that, with increasing of treatment concentration and consequently increasing toxicity, there was an inhibitory effect on cell division. So, cells may be prevented from entering prophase or there may be prophase arrest where cells enter into mitosis but are arrested during prophase, resulting in a high frequency of prophase cells.

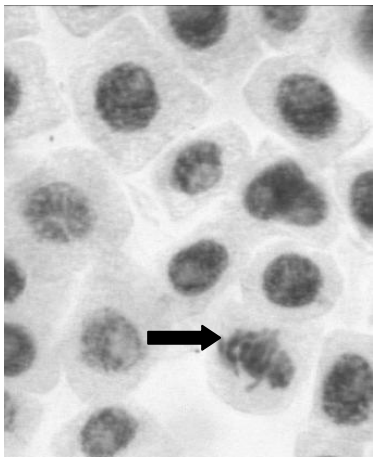
The treatment of *Allium cepa* root tip cells with water extract of *M. nodiflorum* induced three types of chromosomal aberrations. Treatment with water extract of *M. nodiflorum* induced the formation of chromosomal stickiness. Stickiness is attributed to the difficulty of placement of inter chromosomal chromatin fibers or reflects the change in the properties of DNA or its structural protein **(El-Ghamery *et al*., 2003).**

The secondary type of abnormalities was irregular prophase. Irregular prophase was induced in prophase stage after treatment with the different concentrations of the applied extracts. **Kabarity, (1966)** stated that, the induction of irregular prophase might be attributed to the delay or even failure of nuclear membrane to break regularly. This may be the effect of the tested water extract.

Distributed phases were induced after treatment with different doses of *M. nodiflorum* water extract. The induction of distributed phases confirming the suggestion that the effect of plant extract studied was on the mitotic spindle. The plant extract may suppress the spindle formation by interfering with tubulin and lowering the polymerization of microtubular subunits forming the spindle apparatus, thus chromosomes lose their ability to continue from metaphase stage to anaphase and telophase stage, **Pickett-Heaps *et al.,* (1982) and Mukherjee *et al*., (1990).**

5. Conclusion

From this study, it can be concluded that *M. nodiflorum* water extract has a potent inhibitory effect on the mitotic activity of the root tip cells of *Allium cepa*.



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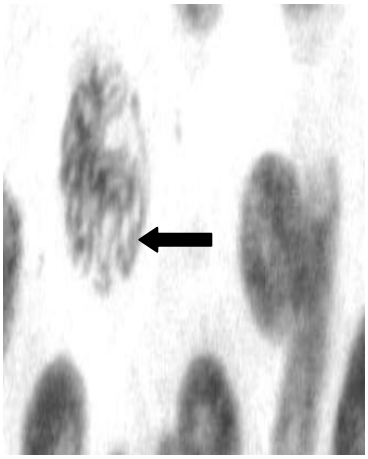

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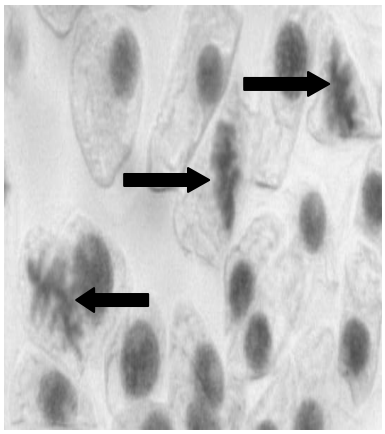

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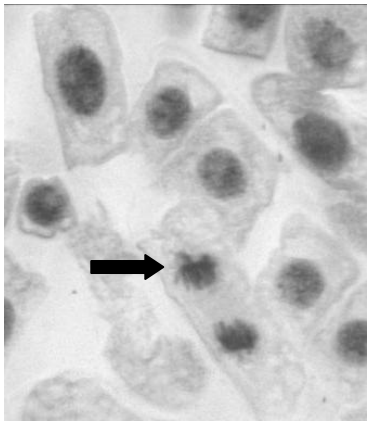

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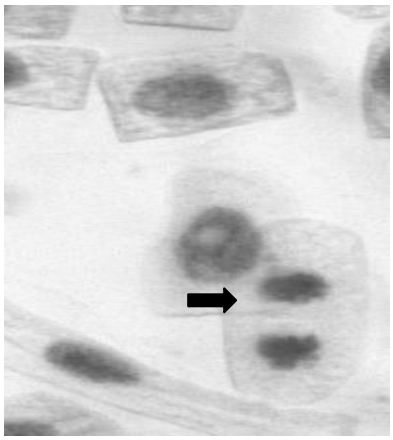

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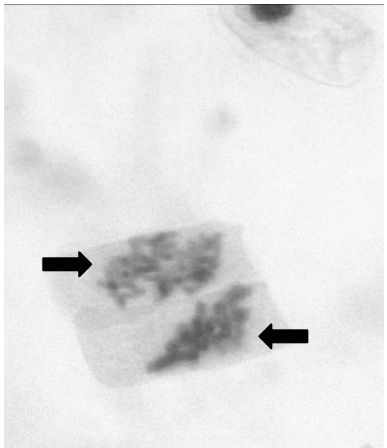

F.

Figure 3. Types of chromosomal aberrations induced in *Allium cepa* meristems treated with water extract of *M. nodiflorum*

(A, B) Irregular prophase after treated with 3% for 6h (C) Sticky metaphase after treated with 1% for 12h (D, E) Sticky anaphase after treated with 0.1% for 3h (F) Disturbed metaphase after treated with 3% for 3h

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الملخص باللغة العربية

دراسة سيتولوجية وكيمياء نباتية على نبات *Mesembryanthemum nodiflorum*

اية محمد عبدالجواد ، سحر عبدالفتاح عبدالتواب ، شيماء سلمي صبيح ، دينا مصطفى فهمي

يعتبر نبات *Mesembryanthemum nodiflorum* من اهم النباتات المستخدمة في الطب الشعبي لعلاج التهابات العين في تونس كما يعتبر احد مصادر الطعام في جنوب افريقيا. تم تجميع العينات النباتية من محمية الغابة المتحجرة بمنطقة شرق القاهرة. ويهدف البحث إلى دراسة التأثير الخلوي للمستخلص المائي لنبات *M. nodiflorum* من خلال معاملة خلايا القمم المرستيمية لجذور نبات البصل بثلاث تركيزات مختلفة 0.1، 1، 3 % عند تعريضها لفترات زمنية مختلفة 3، 6، 12 ساعة.

واوضحت الدراسة انخفاض في معدل الانقسام الميتوزي لخلايا القمم النامية لنبات البصل بعد معاملتها با لتركيزات المختلفة للمستخلص المائي. وكانت اقل قيمة لمعدل الانقسام هى1.35% ووجدت في العينات المعاملة بتركيز 3% بعد مرور 12 ساعة واعلى قيمة لمعدل الانقسام هى 2.68% ووجدت في العينات المعاملة بنفس التركيز 3% بعد مرور 6 ساعات.

واوضحت دراسة كيمياءالنبات وجود مركبات ال alkaloids و triterpenes.