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Article

Cytogenotoxic effect of trichothecene T2 toxin on Allium sativum root tip meristematic cells

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ABSTRACT

Trichothecene T2 is a mycotoxin from the Fusarium species. This research aims to test the effect of the Trichothecene T2 toxin on mitotic index% (M.I.%) and induction of mitotic aberrations by using the Allium sativum (garlic) test system. The toxin concentrations in ppm were 0.00, 0.30, 0.60, 0.90, and 1.20 for 12 hours. The garlic roots were then cut, and mitotic slides were prepared using the squash method and examined under a light microscope. The results revealed that mycotoxin has a significant mitodepressive effect at all concentrations compared to the control, and the MI% reduction was proportional to the increase in toxin concentration. The highest reduction in mitotic index was observed in the 1.2 ppm treatment.

Moreover, this mycotoxin induced and increased the rate of mitotic abnormalities% (MA%) with increasing the mycotoxin concentration. The observed mitotic abnormalities were star-shaped anaphase, sticky metaphase, C-mitosis, sticky anaphase, depolarization, micronuclei, laggard chromosomes, anaphase bridges, and chromosome loss. The least frequently observed abnormality was micronuclei compared to the most frequent aberration, laggard chromosomes. The total mitotic abnormalities significantly increased with increasing the toxin dose concentration. These results suggest that this mycotoxin can inhibit the mitotic activity of the meristematic cells, is mutagenic, and can disrupt the spindle fiber activity of the dividing cells at all concentrations, especially at higher doses in food. Therefore, the foods must be tested for fungi producing this mycotoxin.

Keywords: Mycotoxin; mitodepressive; root tip; spindle fibers; mutagenic.

INTRODUCTION

Mycotoxins are natural chemicals that fungi produce that infect cereals and other agricultural foods. They have a detrimental impact on Animal and human health. They are also thermally stable and easily transmitted to humans through animal products, sophisticated food preparation, and food chains[1]. toxic compounds are produced in foods and feeds by several strains of toxigenic fungi[2], which ultimately cause health problems in humans and animals[3]. Trichothecenes (TCTs) are a family of chemically related mycotoxins produced most commonly by filamentous fungi such as Fusarium, Myrothecium, Stachybotrys, Trichoderma, and Trichothecium, Verticimonosporium, Cylindrocarpon, threatening the health of both animals and human[4,5]. The fungi that produce TCT are found all over the world. Their ability to grow under various environmental conditions, including nutrient, moisture, temperature, and oxygen levels in the growth medium, results in successful colonization[6,7]. T-2 toxin can be found in diverse regions worldwide and negatively affects human and animal health. It affects many organs and organ systems in various ways: neurotoxicity, immunotoxicity, hepatotoxicity, dermal toxicity, nephrotoxicity, as well as reproductive system disruption, maternotoxic and embryo

lethal[8]. T-2 toxin is thought to be a primary contributor in human alimentary toxic aleukia. TCT are non-volatile sesquiterpenoids with low molecular weight (usually 200-500 Da) produced by the terpenoid biosynthesis pathway[9,10]. They are somewhat soluble in water but very soluble in polar organic solvents such ethyl acetate, chloroform, ethanol, methanol, and propylene glycol[1]. TCTs share a three-ring molecule called 12,13-epoxytrichothec-9-ene (EPT) [11,12]. The A-ring cyclohexene is fused to the B-ring tetrahydropyran, which is bridged by a two-carbon chain at C-2 and C-5, generating a cyclopentyl moiety (C-ring)[13]. TCT are classified into four kinds (A-D) based on the substitution pattern of EPT[14]. A hydroxyl (OH) group distinguishes type A TCT.

Define C-8 as having a group, an ester function, or no oxygen substitution[15]. Trichothecenes are quickly absorbed and suppress protein synthesis in growing tissues. Mycotoxins are natural chemicals fungus generate that infect grains and other agricultural goods. Trichothecenes mycotoxin produced by the species Fusarium and Trichoderma is becoming increasingly important in agriculture globally because of the potential health implications. The most dangerous type A trichothecene mycotoxin is T-2 toxin, an unavoidable environmental hazard. T-2 toxin has a considerable detrimental effect on reproduction. Plant systems like onion, garlic and bean are widely used to determine the cytotoxic and genotoxic effects of various chemicals like food additives, and various pesticides, and different toxins[16,17,18]. Trichothecenes are important foodborne mycotoxins implicated in human health and have immune cytotoxic effects. Toxicity data on HT-2 toxin are very limited. To the best of the authors' knowledge, no published data tests the cytogeneotoxic effects of this mycotoxin on A sativum root tip meristematic cells. Therefore, this study aims to test the Cytogenotoxic effect of T-2 mycotoxin on mitotic index and T-2 toxin-induced mitotic aberrations in A. sativum dividing cells at the root apical meristem.

MATERIALS AND METHODS

Garlic heads (Allium sativum, 2n=16) were purchased from a local store in Zakho, free from fertilizers.

Source of the mycotoxin T2 toxin:

Mycotoxin used in the current study is the 8230 Veratox for T-2/HT-2 (NEOGEN company /USA), Cross Reactivity T-2 toxin 100%, HT-2 toxin B 100%. The product details is Veratox® for T-2/HT-2 is a competitive direct ELISA that provides a quantitative analysis of T-2 toxin and HT-2 toxin in commodities such as wheat, rye, barley, oats, and corn. The different concentrations were diluted by using distilled water.

The garlics were placed on tubes filled with distilled water for a few days until the roots were emerged and reached the length of about 1 cm then they were removed from those tubes and placed on another set of tubes with different concentrations of the mycotoxin T2 for about 12 hours. Then, the roots were harvested, and microscopic slides were prepared for the root tip using the squash method. Then, they were stained with Feulgen Giemsa stain and visualized under light microscopy[19].

The mitotic index calculation:

The MI% calculation was done according to the following formula as published by Fiskesjö, (1985).

M.I.%= DC/TC *100 (1)

Where, M.I.= Mitotic Index, DC=Dividing Cells, TC=Total counted Cells.

Mitotic abnormalities percentage:

The mitotic abnormalities percentage was calculated by the following formula (Palsikowski et al., 2017)

MA%= ADC/TC*100 (2)

Where, MA=Mitotic Abnormalities, ADC=Abnormal dividing cells, TC=Total counted cells.

Statistical analysis:

Most of the statistics were done using SPSS (Statistical Package for the Social Sciences) software version 14, by One way ANOVA, and differences between all the treatments were determined by Duncan's multiple-range test [20]. The data were calculated as mean ± standard error. Each experiment was repeated at least three times. The significance was measured at P<0.05. The bar graphs were generated using GraphPad Prism version 9.0.

RESULTS

Trichothecenes are a class of structurally related mycotoxins with different cytotoxicity degrees. Because of the potential health risks, trichothecene (TCT) mycotoxin is becoming increasingly important in agriculture around the globe. It is primarily metabolized and eliminated after consumption, producing more than 20 metabolites, the most important of which is the hydroxy trichothecenes-2 toxin. (A12). The effect of T2 toxin on mitotic index% is shown in Table 1 and Figure, 1.

Treatments (ppm)	Time (hours)	Total no. of cells	interphase	Dividing cells	Mitotic index%
0.00	12	1127.000 ± 23.643	1009.400 ±19.954 a, b	117.600 ± 4.345 a	10.4300 ± 0.222 a
0.30	12	990.000 ±45.655	915.928 ±42.771 b	74.072 ± 2.942 b	7.4867 ± 0.075 b
0.60	12	1102.000 ± 56.580	1049.593 ±53.323 a	52.407 ± 3.311 c	4.75 ± 0.078 c
0.90	12	1052.333 ±26.194	1013.816 ±25.189 a, b	38.518 ± 1.073 d	3.68 ± 0.036 d
1.20	12	996.667 ±3.844	978.223 ±4.019 a, b	18.444 ± 0.939 e	1.851 ± 0.095 e
Significance			n.s.	***	***

[1] Note: n.s. means non-significant, *** means significant at p<0.001. DMRT produced different superscript letters.

Table 1: The effect of T2 toxin on the mitotic index% of *A. sativum* root tip cells.

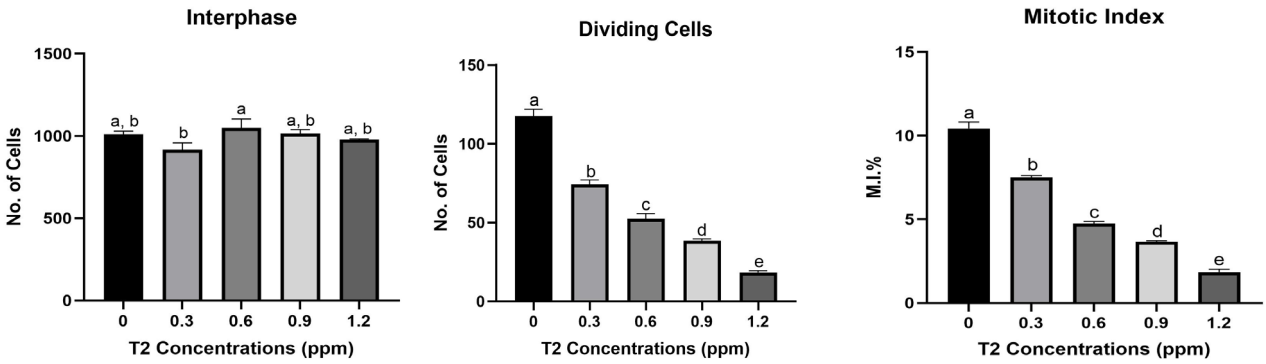

Figure 1: The effect of T2 toxin on no. of cells in interphase and dividing cells, and mitotic index% of *A. sativum* root tip cells.

The highest concentration, 1.5 ppm which caused it to decrease from 10.53 in the control to 0.7633 at 1.5 ppm. The mitodepressive effect was significant at P<0.001.

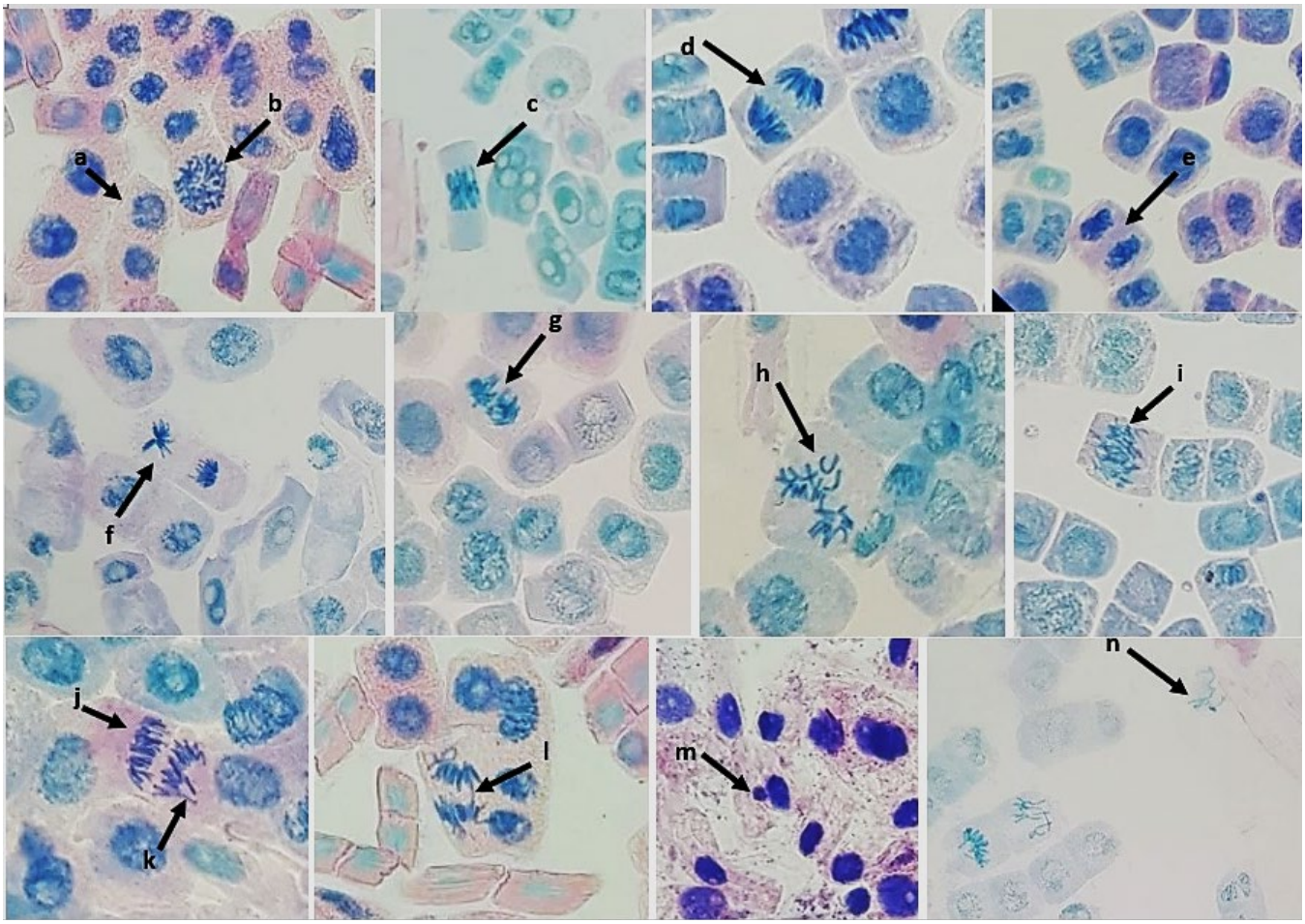

Figure 2: Normal mitosis and different abnormalities in A sativum root apical meristematic cells: a-normal interphase, b- normal prophase, c- normal metaphase, d- normal anaphase, e- normal telophase, f- Star-shaped anaphase, g- Sticky Metaphase, h- C-Mitosis, i- Sticky anaphase, j- Depolarization, k- Laggard Chromosomes, l- Anaphase Bridge, m- Micronucleus, n- Chromosome Loss.

The effect of the T2 toxin on chromosomal aberrations is shown in Table 2 and Figure 3. According to the Table, the aberrations increased proportionally with increasing mycotoxin concentration. Total mitotic abnormalities increased proportionally with the increase in the concentration of mycotoxin. The observed mitotic abnormalities were star-shaped anaphase, sticky metaphase, C-mitosis, sticky anaphase, depolarization, micronuclei, laggard chromosomes, anaphase bridges, and chromosome loss. The least frequently observed abnormality was micronuclei at 0.00 in the control and increased to 0.1672 in the 1.2 ppm of T2. Compared to the most frequent aberration, laggard chromosomes were 0.0536% in the control and increased to 1.1341% in 1.2 ppm of the tested mycotoxin. The total CAI% was 0.1106 in the control group, which increased to about 5.2168% at the highest concentration of the mycotoxin. Significant differences existed between treatments at $p<0.001$ for all mitotic aberrations.

T2 concentration (ppm)	Star shaped anaphase%	Sticky metaphase%	C-Mitosis%	Sticky anaphase%	Depolarization%	Laggard Chromosomes%	Anaphase bridges%	Micronuclei%	Chromosome loss%	Total CA%

0.0	0.000 ± 0.000 d	0.0000 ± 0.000 d	0.0571 ± 0.022 c	0.0000 ± 0.000 e	0.000 ± 0.000 d	0.0536 ± 0.0192 5 e	0.000 ± 0.000 c	0.000 ± 0.000 c	0.000 ± 0.000 e	0.1106 ± 0.03676 d
0.3	0.0761 ± 0.011 c	0.1175 ± 0.008 c	0.5833 ± 0.039 b	0.1910 ± 0.027 d	0.245 ± 0.030 c	0.2406 ±0.035 18 d	0.004 ± 0.004 c	0.000 ± 0.000 c	0.108 ± 0.003 d	1.5644 ±0.0815 8 c
0.6	0.100 ± 0.005 c	0.4700 ± 0.041 a	1.0310 ± 0.058 a	0.3741 ± 0.012 c	0.287 ± 0.025 b, c	0.4142 ± 0.0029 0 c	0.079 ± 0.008 b	0.0000 ± 0.000 c	0.174 ± 0.0120 c	2.9284 ±0.1397 5 b
0.9	0.1869 ± 0.008 b	0.2466 ± 0.027 b	0.6432 ± 0.005 b	0.5285 ± 0.019 b	0.358 ± 0.016 b	0.6268 ± 0.0201 9 b	0.085 ± 0.0074 2 b	0.1112 ± 0.013 b	0.387 ± 0.004 b	3.1728 ±0.0207 7 b
1.2	0.2802 ± 0.010 a	0.4619 ± 0.031 a	0.9601 ± 0.033 a	0.8026 ± 0.015 a	0.532 ± 0.034 a	1.1341 ± 0.0397 2 a	0.197 ± 0.010 a	0.1672 ± 0.016 a	0.682 ± 0.039 a	5.2168 ±0.0526 3 a
F value	192.205	62.803	116.06 4	329.04 3	63.116	240.15 3	142.99 0	70.784	212.055	591.802
Significance	***	***	***	***	***	***	***	***	***	***

*** means significant at p<0.001. DMRT produces superscript letters

Table 2: the effect of T2 toxin on mitotic abnormalities% of A. sativum root tip meristematic cells after 12 hours.

The abnormalities caused by this mycotoxin in A. sativum root tip meristematic cells were star-shaped anaphase, sticky metaphase, C-mitosis, sticky anaphase, depolarization, micronuclei, and laggard Chromosomes. The most frequent abnormalities were C-mitosis and sticky anaphase. According to Table 2, the total abnormalities increased with the increase in the toxin concentration. The highest number was observed with the 1.5 ppm treatment. The different abnormalities and normal mitosis are shown in Figure 2. The mycotoxin T2 induces the distortion and inhibition of mitotic spindle formation, which causes the C-mitotic cells and stickiness in metaphase and anaphase. Moreover, it induces chromosome loss and micronuclei.

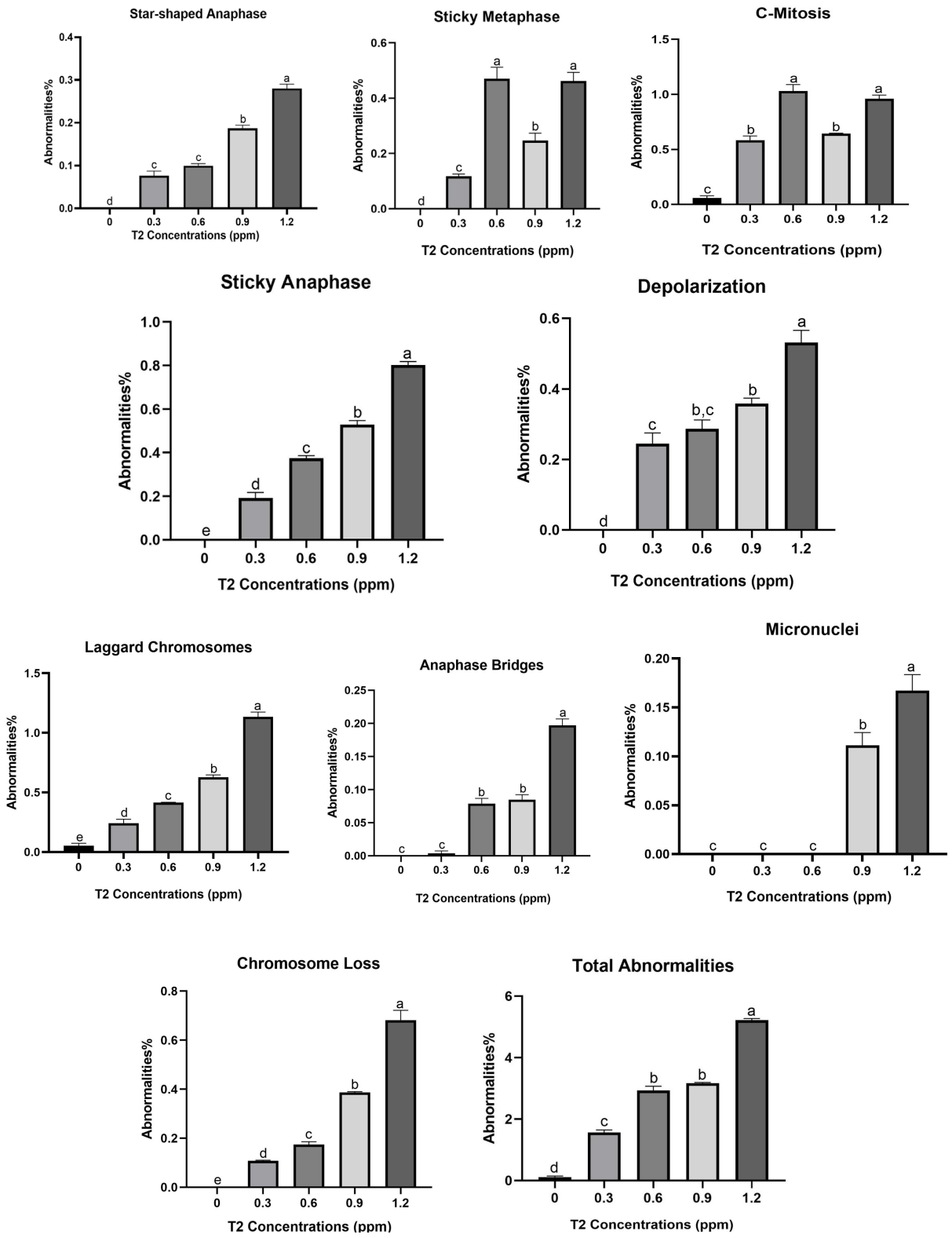

Figure 3: The effect of T2 toxin on mitotic abnormalities in A. sativum root tip meristematic cells.

About 1000 cells were counted for each slide and each experiment was repeated thrice. According to table 1, the different concentrations of the mycotoxin caused a significant reduction in the mitotic index at all treatments along with the reduction in the dividing cells number. However, the greatest effect on the mitotic index was observed at the highest concentration of the mycotoxin treatment (1.2 ppm) which caused the mitotic

index% to decrease from (10.4300 ± 0.222) in the control group to (1.851 ± 0.095) at 1.2 ppm treatment. The mitodepressive effect was significant at P<0.001. Moreover, there were significant differences among the treatments as detected by DMRT.

The effect of the T2 toxin on chromosomal aberrations is shown in Table 2 and Figure 3. The abnormalities caused by this mycotoxin in A. sativum root tip meristematic cells were star-shaped anaphase, sticky metaphase, C-mitosis, sticky anaphase, depolarization, micronuclei, and laggard chromosomes. The most frequent abnormalities were C-mitosis and sticky anaphase. According to Table 2, the total abnormalities proportionally increased with increasing toxin concentration. The highest number was observed with the 1.2 ppm treatment. The different abnormalities and normal mitosis are shown in Figure 2.

The total MA% was (0.1106 ± 0.03676) in the control group, which increased to about (5.2168 ±0.05263) at the highest concentration of the mycotoxin used. Significant differences existed between treatments at p<0.001 for all mitotic aberrations. The ability to induce different abnormalities in the A. sativum cells at the root apical meristem indicates its mutagenic activity.

DISCUSSION

According to Table 1 the different concentrations of mycotoxin caused a significant reduction in the mitotic index at all treatments. However, the greatest reduction in the mitotic index was observed at other research which tested the effect of Aflatoxin B1 induces reactive oxygen species-dependent caspase-mediated apoptosis in normal human cells, inhibits Allium cepa root cell division [21], and another research tested the toxic activity of citrinin, a fungal phytotoxin, and its mode of action in onion cells [22].

The abnormalities caused by this mycotoxin in A. sativum root tip meristematic cells were star-shaped anaphase, sticky metaphase, C-mitosis, sticky anaphase, depolarization, micronuclei, and laggard Chromosomes. The most frequent abnormalities were C-mitosis and sticky anaphase. According to Table 2, the total abnormalities increased with the increase in the toxin concentration. The highest number was observed with the 1.5 ppm treatment. The different abnormalities and normal mitosis, are shown in Figure 2. The mycotoxin T2 induces the distortion and inhibition of mitotic spindle formation, which causes the C-mitotic cells and stickiness in metaphase and anaphase. Moreover, it induces chromosome loss and micronuclei.

The ability to induce different abnormalities in the A. sativum cells at the root apical meristem indicates its mutagenic activity. The induction of both Micronuclei and stickiness are the most obvious indicators of cytotoxicity. According to Table 2, the increase in the frequency of micronuclei was dose-dependent. These results are in agreement with the results obtained by [23]. The total abnormality percentage increase was also dose-dependent. This increase agrees with previous results of testing various substances on onion root tips [24,25].

These results are in agreement with other research which tested the effect of Aflatoxin B1 on reactive oxygen species-dependent caspase-mediated apoptosis in normal human cells, and on inhibition of Allium cepa root cell division [26], and another research tested the toxic activity of citrinin, a fungal phytotoxin, and its mode of action in onion cells [27]. The mycotoxin T2 induces the distortion and inhibition of mitotic spindle formation which causes the C-mitotic cells and stickiness in metaphase and anaphase. Moreover, it induces chromosome loss and micronuclei. The least frequently observed abnormality was micronuclei at (0.000 ± 0.000) in the control and increased to (0.1672 ± 0.016) in the 1.2 ppm of T2. On the other hand, the most frequent aberration observed was laggard chromosomes were (0.0536 ± 0.01925) in the control and increased to (1.1341 ± 0.03972) in 1.2 ppm of the tested mycotoxin. A laggard chromosome is defined as a chromosome that did not overlap along the long axis of the spindle with any of the properly segregating chromosomes [28]. The induction of both Micronuclei and stickiness are the most obvious indicators of cytotoxicity, and Micronuclei are small membrane-bounded compartments with a DNA content encapsulated by a nuclear envelope and spatially separated from the primary nucleus (Krupina et al., 2021). Chromosome stickiness may result from chromatin fibers' sticking to each other or breaking due to erroneous or inadequate condensation of these fibers, as a consequence of this, movement of mitotic spindle fibers together with inner-chromosome stickiness when the chromosome is drawn to the pole causes secondary anomalies (bridge and fragment occurrence) , Stickiness in chromosomes is an indication of the high toxicity of the chemical substance and usually this may kill the cells with the irreversible damages [25]

CONCLUSIONS

The mycotoxin T2 is a mutagen that inhibits cell division and induces a wide range of abnormalities in A sativum root apical meristematic cells at lower doses. It is very strong. Therefore, the food grains that contain this mycotoxin should be tested carefully to ensure that they are not contaminated with the T2 toxin-producing fungi to avoid its hazardous health risks.

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