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# Research Article
# Cytotoxicity Effects of Biological Control and Antioxidants using *Vicia faba* Chromosomal Aberration Assay

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## Abstract

**Background and Objective:** Biological control refers to purposeful utilization of introduced or resident living organisms other than disease resistant host. Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage. Cytological abnormalities in *Vicia faba*, studies are carried out in root tips as the using of this area in induction of chromosomal aberrations is one of the oldest, simplest, most reliable and inexpensive method. Therefore, this study aims to determine the cytotoxic and mutagenic effect using chromosome aberration assay (*Vicia faba*). **Materials and Methods:** *Vicia faba* (broad bean) seeds (2n = 12 chromosome) (Cultivar: Giza 716), were obtained from the Agriculture Research Center, Crop Research Section, Sakha, Kafr El-Sheikh, Egypt. The seeds were prepared into slides via serial steps of fixation and squashing. Data of different treated groups of root tips were represented as Mean±SE and statically analyzed using t-test to determine the significance of the differences between treatments and control at the 0.05 level of probability. **Results:** Results showed the highest percentage of MI% was 23.77% at 20 mM of citric acid for 24 h, while the lowest percentage was 9.54% at $H_2O$+*Trichoderma viride* for 48 h. The highest percentage of abnormal mitosis was 43.53 and 37.24% at 24 h for 20 mM of (Aa+*T. harzianum*) and 3 mM of salicylic acid, respectively compared with control sample 9.37%, while the highest abnormal mitosis percentage at 48 h was 64.93 and 43.13% for ($H_2O$+*T. viride*) and 20 mM citric acid. **Conclusion:** It was concluded that *Trichoderma* can be used safely as bio-control agents against pathogenic microbes. To use bioagents like antioxidants especially ascorbic acid and bio-control fungi especially *T. harzianum* in future in replacement of chemical fungicides (unsafe and inexpensiveness).

Key words: Cytotoxicity effects, biological control, antioxidants, *Vicia faba*, mitotic index, *Trichoderma harzianum*, *Trichoderma viride*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Biological control is considered as a friendly environmental method for controlling plant disease and more safe, available and economical alternative to some insecticides, pesticides and fungicides[1]. Biological control refers to purposeful utilization of introduced or resident living organisms other than disease resistant host plants to suppress the activities and populations of one or more plant pathogens[2]. Defined as the use of natural or modified organisms, genes or gene products to reduce effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects and microorganisms[3].

The use of *Trichoderma* spp. as a biological control agent for plant disease for over 70 years[4] as it can destroy total or partial plant pathogen populations[5] *Trichoderma* strains become the most studied and applied biocontrol agent[6] as it can control growth of deuteromycetous, ascomycetous and basidiomycetous fungi, which are soil and air-borne fungi[7].

Antioxidants found to be safe on environment, human and plant, it had been used for controlling of some diseases such as root and pod rot in peanut[8], root rot and leaf blight in lupine[9]. Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage[10]. Antioxidants especially salicylic acid becomes the new opportunity in controlling plant diseases caused by fungi or bacteria, in the same time low in cost and increases crop protection by enhancing plant resistance to pathogen[11].

Cytological abnormalities in *Vicia faba*, studies are carried out in root tips as the using of this area in induction of chromosomal aberrations is one of the oldest, simplest, most reliable and inexpensive method[12].

Ma[13] established *Vicia faba* root tip micronucleus test as this way is efficient test to detect very weak mutagenic effects and clastogenicity by different hazardous pollutants which affect human health. *Vicia faba* is considered a good testing plant as its chromosomes is nearly like human lymphocytes in sensitivity to the same mutagen[14] and also can be used in detecting clastogenic potential of antioxidant and antimicrobial substances[15]. Plants are widely used in detection of any chromosomal abnormalities resulted from environmental pollution or heavily use of fungicides as their chromosomal organization especially in root tips like that in human and also mitosis and meiosis in plant cell is like those of human or animal[16].

This study aims to investigate the cytological effect of *Trichoderma viride*, *Trichoderma harzianum* and selected antioxidants (salicylic acid, citric acid and ascorbic acid) by using *Vicia faba* plant bioassay. The measured parameters include mitotic index, phase index and chromosome aberrations.

## MATERIALS AND METHODS

**Sample collection:** The seeds of *Vicia faba* var. Giza 716 obtained from The Agricultural Research Center, Crop Research Section, Sakha, Kafr El-Sheikh, Egypt, 2015.

**Chromosomal aberration assay:** The seeds of *Vicia faba* treated with nine treatments as shown in Table 1. All treatments applied for two different exposure times 24 and 48 h. The root tips were fixed in carny's solution (1 glacial acetic acid: 3 ethanol ratio) and stored in refrigerator atleast for 48 h. The root tips were hydrolyzed in 1N HCl at 60°C for 3 min the root tips were stained using a double staining method combining the modified carbol fuchsin reaction[17,18], where the root tips were put in carbol fuchsin overnight then in 2% aceto-orcein stain for 2 h[19]. The mitotic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed. At least 2000 cells from 20 slides of each treatment were examined. The cells were recorded as normal or aberrant in the different stages of mitotic division: Interphase, prophase, metaphase, anaphase and telophase. All cells with aberration were counted and photographed using Olympus camera (SC35 type 12 mode).

**Statistical analysis:** Data of different treated groups of root tips were represented as Mean±SE (Standard error) and statically analyzed using t-test to determine the significance of the differences between treatments and control at the 0.05 level of probability according to Snedecor and Cochran[20].

Table 1: Treatments by antioxidants and *T. harzianum* and *T. viride* in *Vicia faba*

| Antioxidants | Combinations |
|---|---|
| Salicylic acid (SA) | 1,3,5 and 7 mM |
| Citric acid (CA) | 5,10,15 and 20 mM |
| Ascorbic acid (AA) | 5,10,15 and 20 mM |
| Combination 1 | SA: 3 mM+*T. viride* |
| Combination 2 | CA: 20 mM+*T. viride* |
| Combination 3 | AA: 20 mM+*T. viride* |
| Combination 4 | SA: 3 mM+*T. harzianum* |
| Combination 5 | CA: 20 mM+*T. harzianum* |
| Combination 6 | AA: 20 mM+*T. harzianum* |

## RESULTS AND DISCUSSION

Biological control with bio-control agents found to be safe on the environment with less harmful effects on all organisms, low in cost, easily to be obtained and also available in nature or can be collected. It can be defined as using natural organisms or genetically modified or gene products to reduce the effect of undesired pathogen on the favor useful organisms to human such as crops, trees, animal and useful organisms[21].

Antioxidants have a strong antimicrobial action due to its ability to inhibit the function of enzymes by oxidized compounds, dissolve membrane lipids and interfere with nutrients transportation or synthesis of DNA, RNA and protein[22].

Cytological studies are considered as important signs for harmful effects which appeared on infected plants treated with chemical fungicides[23]. There is another definition to biological control related with the cytological study and it states that "the development of transgenic plants and biologically induced systemic resistance in hosts[3]". So, it was necessary to study the cytological effect of the tested biological control agents to a strategic plant such as *Vicia faba.* Cytological studies are considered as important signs for harmful effects which appeared on infected plants treated with chemical fungicides[23].

The effect of different antioxidants on mitotic indices (MI%), phase indices (PI%), types and total abnormalities (Tab%) are given in Table (2-5). There is significant increase in mitotic index at majority of the treatments for exposure time 24 and 48 h. It was demonstrated at Table (2, 3), the highest percentage of MI% was 23.77% at 20 mM of citric acid for 24 h, while the lowest percentage was 9.54% at $H_2O$ + *T. viride* for 48 h.

For treatments at exposure time 24 h the lowest percentage of MI% was (11.25%) at com. (AA: 20 mM+ *T. harzianum*) when compared to the highest value which was (23.77%) at 20 mM of citric acid.

Also there is a significant increase in the mitotic index for treatments at exposure time 48 h as the highest value was (22.88%) at 3 mM of salicylic acid combined with *T. harzianum* compared to control value (12.26%). When mitotic index is higher than the control is the result of increasing in cell division that is harmful to the cell. Also the using of such treatment induced slightly decrease in mitotic index values. This reduction may be due to increasing in interphase duration that inhibit DNA synthesis and increasing in G1 phase duration[24] and these results are seemed to be like those which proved by Liu *et al.*[25]. It was proved that changes in mitotic index (increase or decrease) can be used in determination of cytotoxicity levels of an agent and can be used as a monitor for environmental pollution[26,27].

Results obtained showed that there was an increase or decrease in percentages of prophase, metaphase, anaphase and telophase stages when compared to control. That may be due to the using of different treatments with different durations of each stage of mitosis[28].

It was observed that there was some differences in the prophase stage ranged from increase and decrease in values compared to control. The highest frequency of prophase was (33.86%) at 20 mM of ascorbic acid for 48 h. The lowest frequency of prophase was found to be at value (11.41%) at com. ($H_2O$+*T. harzianum*) for 24 h compared to control values (14.84 and 25.52%) for 48 and 24 h, respectively. In the same manner, it was found to be slight increase and decrease in values of metaphase. The lowest value for exposure time 24 h was (25.78%) for seeds treated with (CA: 20 mM+*T. harzianum*) and the highest value for the same exposure time was (47.5%) for treatment ($H_2O$+*T. viride*) compared to the control value at 24 h which was (35.57%). On the other hand, the frequency in anaphase stage showed that there is a large difference in values of treated seeds with the antioxidants and their combinations with *T. harzianum* or with *T. viride* represented in the percentages of increasing and decreasing when compared to control. The highest frequency value was (31.04%) at com. (SA: 3 mM+*T. viride*) and the lowest value was (5.31%) at com. ($H_2O$+*T. viride*) for exposure time 24 h compared to control (13.02).

Finally, at telophase stage the highest value was (32.98%) at com. ($H_2O$+*T. harzianum*) and the lowest value was (18.64%) at 3 mM of salicylic acid for exposure time 24 h when compared to the control (25.89%).The telophase frequency for exposure time 48 h as the control value was (42.08%) where the maximum value was (32.98%) for com. ($H_2O$+*T. harzianum*) and the minimum value was (13.49%) for 3 mM of salicylic acid combined with *T. viride*. Increase in metaphase, anaphase and telophase values was observed in contrast to prophase stage. This may be due to the effect of used treatments on the spindle in the arrest of division at these stages and these results are in agreement with those obtained by Badr[29] and Selim[30] and in squid pens chitosan on *Vicia faba*[31].

The production of chromosomal abnormalities by chemical compounds is regarded as a reliable evidence of the genotoxocity[12]. In the present study, the abnormalities were recorded namely; micronucleus cells at interphase stage. Stickness, non-congression, ring, two groups, star and disturbed at metaphase stage, late separation, diagonal,

Table 2: Mitotic index, normal and abnormal phase indices and total abnormalities in non-dividing and dividing cells after treating *Vicia faba* root tips which treated with antioxidants and their combinations with *Trichoderma*

| Treatments | | | Phase index | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Prophase (%) | | Metaphase (%) | | Anaphase (%) | | Telophase (%) | | Total abnormal (%) | |
| Concn. | ET | MI (%) | Mitotic | Abnormal | Mitotic | Abnormal | Mitotic | Abnormal | Mitotic | Abnormal | Interphase | Mitosis |
| Control $H_2O$ | 24 | 11.06±0.85 | 25.52 | 0 | 35.57 | 6.77 | 13.02 | 0.00 | 25.89 | 2.60 | 0.00±0.00 | 9.37±3.79* |
| | 48 | 12.26±1.05 | 14.84 | 0 | 25.47 | 14.59 | 17.61 | 0.78 | 42.08 | 3.13 | 0.00±0.00 | 18.50±5.54 |
| AA | 24 | 17.77±1.49* | 23.91 | 0 | 31.35 | 14.69 | 19.22 | 5.68 | 25.52 | 10.16 | 0.00±0.00 | 30.53±5.51* |
| | 48 | 16.25±0.85* | 33.86 | 0 | 27.86 | 7.71 | 11.59 | 4.79 | 26.69 | 5.47 | 0.00±0.00 | 17.97±2.78ns |
| SA | 24 | 15.59±1.31* | 19.38 | 0 | 37.40 | 21.62 | 24.58 | 8.85 | 18.64 | 6.77 | 0.16±0.16ns | 37.24±5.85* |
| | 48 | 16.83±0.88* | 24.11 | 0 | 22.03 | 19.01 | 24.90 | 1.67 | 28.96 | 1.56 | 0.00±0.00 | 22.24±2.73ns |
| CA | 24 | 23.77±1.27* | 25.74 | 0 | 38.53 | 9.33 | 11.91 | 2.86 | 23.82 | 3.05 | 0.00±0.00 | 15.24±2.53* |
| | 48 | 17.85±1.49* | 14.53 | 0 | 40.52 | 20.78 | 20.83 | 15.11 | 24.11 | 7.24 | 0.00±0.00 | 43.13±4.63* |
| $H_2O$+*T. harzianum* | 24 | 11.25±0.93ns | 20.11 | 0 | 32.92 | 20.31 | 17.03 | 13.14 | 29.93 | 10.08 | 0.00±0.00 | 43.53±5.07* |
| | 48 | 13.54±1.20ns | 14.33 | 0 | 36.67 | 14.43 | 16.02 | 9.11 | 32.98 | 11.38 | 0.00±0.00 | 34.92±5.92* |
| $H_2O$+*T. viride* | 24 | 17.91±0.78* | 20.16 | 0 | 47.50 | 19.27 | 5.31 | 0.00 | 27.03 | 9.90 | 0.00±0.00 | 29.17±3.39* |
| | 48 | 9.54±1.15* | 16.82 | 0 | 33.83 | 28.38 | 17.94 | 17.17 | 31.41 | 19.38 | 0.00±0.00 | 64.93±8.32* |

Et: Exposure time (h), MI: Mitotic index, *: Significant at 0.05, ns: Not significant at 0.05, ±Standard Error

Table 3: Mitotic index, normal and abnormal phase indices, total abnormalities in non-dividing and dividing cells after treating *Vicia faba* root tips which treated by combination antioxidants with *Trichoderma*.

| Treatments | | | Phase index | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Prophase (%) | | Metaphase (%) | | Anaphase (%) | | Telophase (%) | | Total abnormal (%) | |
| Concn. | ET | MI (%) | Mitotic | Abnormal | Mitotic | Abnormal | Mitotic | Abnormal | Mitotic | Abnormal | Interphase | Mitosis |
| AA+*T. harzianum* | 24 | 11.25±0.93ns | 20.11 | 0 | 32.92 | 20.31 | 17.03 | 13.14 | 29.93 | 10.08 | 0.00±0.00 | 43.53±5.07* |
| | 48 | 13.27±0.81ns | 20.05 | 0 | 27.08 | 15.63 | 28.91 | 13.80 | 23.96 | 11.72 | 0.00±0.00 | 41.15±3.47* |
| AA+*T. viride* | 24 | 17.23±1.07* | 25.10 | 0 | 30.83 | 11.72 | 19.38 | 7.55 | 24.69 | 14.17 | 0.00±0.00 | 33.44±1.92* |
| | 48 | 16.94±0.88* | 21.51 | 0 | 36.02 | 16.41 | 20.10 | 7.29 | 22.37 | 3.39 | 0.00±0.00 | 27.09±4.10ns |
| SA+*T. harzianum* | 24 | 17.75±0.98* | 19.74 | 0 | 32.55 | 15.73 | 26.67 | 12.44 | 21.04 | 5.58 | 0.00±0.00 | 33.75±2.92* |
| | 48 | 22.88±1.11* | 18.96 | 0 | 27.34 | 9.07 | 28.02 | 11.45 | 25.68 | 3.64 | 0.00±0.00 | 24.16±2.76ns |
| SA+*T. viride* | 24 | 17.59±0.88* | 21.04 | 0 | 34.43 | 11.24 | 31.04 | 10.62 | 13.49 | 9.89 | 0.00±0.00 | 31.75±2.73* |
| | 48 | 17.85±1.49* | 14.53 | 0 | 40.52 | 20.78 | 20.83 | 15.11 | 24.11 | 7.24 | 0.00±0.00 | 43.13±4.63* |
| CA+*T. harzianum* | 24 | 18.43±1.25* | 25.94 | 0 | 25.78 | 10.00 | 18.18 | 8.65 | 30.10 | 15.88 | 0.00±0.00 | 34.53±2.73* |
| | 48 | 12.60±0.68ns | 24.11 | 0 | 33.46 | 15.21 | 18.42 | 0.00 | 24.01 | 4.53 | 0.00±0.00 | 19.74±4.19ns |
| CA+*T. viride* | 24 | 16.61±0.72* | 26.54 | 0 | 33.82 | 11.98 | 19.64 | 10.94 | 20.00 | 4.95 | 0.00±0.00 | 27.87±2.44* |
| | 48 | 17.95±0.89* | 24.84 | 0 | 22.55 | 5.05 | 33.75 | 18.64 | 18.85 | 0.00 | 0.00±0.00 | 23.69±2.50ns |

Et: Exposure time (h), MI: Mitotic index, *: Significant at 0.05, ns: Not significant at 0.05, ±Standard Error

Table 4: Types of chromosome abnormalities and its percentage in each phase in *Vicia faba* root tips treated with antioxidants and their combinations with *Trichoderma*

| Treatments | | | Metaphase | | | | | | | Anaphase | | | | | Telophase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concn. | ET | Interphase Micronucleus | Stickiness | Non-Congression | Two groups | Bring | Micronucleus | Star-metaphase | Disturbed | Late separation | Diagonal | Bridge | Laggard | Disturbed | Bridge | Laggard | Diagonal | Late separation | Disturbed |
| Control $H_2O$ | 24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 6.77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.60 | 0.00 |
| | 48 | 0.00 | 4.17 | 1.56 | 0.00 | 0.00 | 3.13 | 0.00 | 5.73 | 0.00 | 0.00 | 0.00 | 0.00 | 0.78 | 0.00 | 0.00 | 0.00 | 3.13 | 0.00 |
| AA | 24 | 0.00 | 1.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 13.39 | 0.52 | 4.53 | 0.00 | 0.00 | 0.63 | 0.00 | 1.04 | 0.00 | 3.65 | 5.47 |
| | 48 | 0.00 | 4.90 | 0.00 | 0.78 | 0.00 | 0.00 | 0.00 | 2.03 | 0.00 | 3.75 | 0.00 | 0.00 | 1.04 | 0.00 | 0.00 | 0.00 | 1.04 | 4.43 |
| SA | 24 | 0.16 | 4.69 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 16.93 | 5.21 | 1.04 | 0.00 | 0.00 | 2.60 | 0.00 | 0.00 | 1.56 | 4.17 | 1.04 |
| | 48 | 0.00 | 4.69 | 4.17 | 0.52 | 0.00 | 0.00 | 1.56 | 8.07 | 0.00 | 0.00 | 0.00 | 1.04 | 0.63 | 0.00 | 0.00 | 0.00 | 1.56 | 0.00 |
| CA | 24 | 0.00 | 4.90 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.43 | 2.86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.78 | 0.78 | 1.49 |
| | 48 | 0.00 | 8.44 | 2.60 | 1.15 | 0.00 | 0.00 | 0.00 | 8.59 | 3.13 | 0.00 | 0.00 | 0.00 | 11.98 | 0.00 | 0.00 | 0.00 | 6.51 | 0.78 |
| $H_2O$+*T. harzianum* | 24 | 0.00 | 3.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.30 | 2.34 | 0.00 | 0.00 | 0.00 | 6.77 | 0.00 | 0.63 | 1.03 | 5.21 | 5.99 |
| | 48 | 0.00 | 3.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.30 | 2.34 | 0.00 | 0.00 | 0.00 | 6.77 | 0.00 | 0.63 | 0.00 | 5.21 | 5.99 |
| $H_2O$+*T. viride* | 24 | 0.00 | 2.60 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 16.67 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 9.90 | 0.00 |
| | 48 | 0.00 | 7.81 | 0.00 | 3.91 | 7.81 | 0.00 | 0.00 | 8.85 | 3.13 | 0.00 | 0.00 | 4.17 | 9.87 | 0.00 | 0.00 | 3.76 | 14.06 | 1.56 |

ET: Exposure time (h)

Table 5: Types of chromosome abnormalities and its percentage in each phase in *Vicia faba* root tips treated with antioxidants and their combinations with *Trichoderma*

| Treatments | | | Metaphase | | | | | | | Anaphase | | | | | Telophase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concn. | ET | Interphase Micronucleus | Stickiness | Non-Congression | Two groups | Bring | Micronucleus | Star-metaphase | Disturbed | Late separation | Diagonal | Bridge | Laggard | Disturbed | Bridge | Laggard | Diagonal | Late separation | Disturbed |
| AA+*T. harzianum* | 24 | 0.00 | 4.95 | 4.69 | 4.17 | 1.56 | 0.00 | 0.00 | 4.95 | 1.67 | 3.13 | 3.13 | 4.17 | 1.04 | 1.04 | 1.04 | 8.00 | 8.00 | 0.00 |
| | 48 | 0.00 | 3.91 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.72 | 4.69 | 1.04 | 0.00 | 0.00 | 8.07 | 0.00 | 0.00 | 3.38 | 3.65 | 4.69 |
| AA+*T. viride* | 24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.72 | 4.43 | 0.00 | 1.56 | 0.00 | 1.56 | 0.00 | 0.00 | 0.96 | 9.11 | 4.01 |
| | 48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 16.41 | 3.91 | 0.00 | 2.34 | 0.00 | 1.04 | 0.00 | 0.00 | 0.00 | 3.39 | 0.00 |
| SA+*T. harzianum* | 24 | 0.00 | 6.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 9.48 | 5.42 | 1.82 | 0.00 | 2.60 | 2.60 | 0.00 | 0.00 | 0.00 | 1.41 | 4.17 |
| | 48 | 0.00 | 3.13 | 1.15 | 0.00 | 0.00 | 0.00 | 0.00 | 4.79 | 1.56 | 2.34 | 0.00 | 0.78 | 6.77 | 0.00 | 0.00 | 0.00 | 1.82 | 1.82 |
| SA+*T. viride* | 24 | 0.00 | 2.66 | 0.00 | 3.13 | 0.00 | 0.00 | 0.00 | 12.08 | 3.13 | 0.63 | 0.00 | 1.04 | 4.43 | 0.00 | 0.00 | 1.56 | 1.56 | 0.00 |
| | 48 | 0.00 | 2.60 | 2.34 | 0.00 | 0.00 | 0.00 | 0.00 | 6.35 | 4.01 | 0.00 | 0.00 | 0.00 | 6.61 | 1.04 | 1.04 | 0.00 | 7.03 | 0.78 |
| CA+*T. harzianum* | 24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.00 | 0.00 | 0.00 | 0.00 | 0.00 | 8.65 | 0.0 | 0.00 | 0.00 | 13.80 | 2.08 |
| | 48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 15.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 4.53 | 0.00 |
| CA+*T. viride* | 24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 11.98 | 5.21 | 2.60 | 0.00 | 0.00 | 3.13 | 0.00 | 0.00 | 0.00 | 2.34 | 2.60 |
| | 48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.05 | 12.81 | 0.00 | 0.00 | 0.00 | 5.83 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

ET: Exposure time (h)

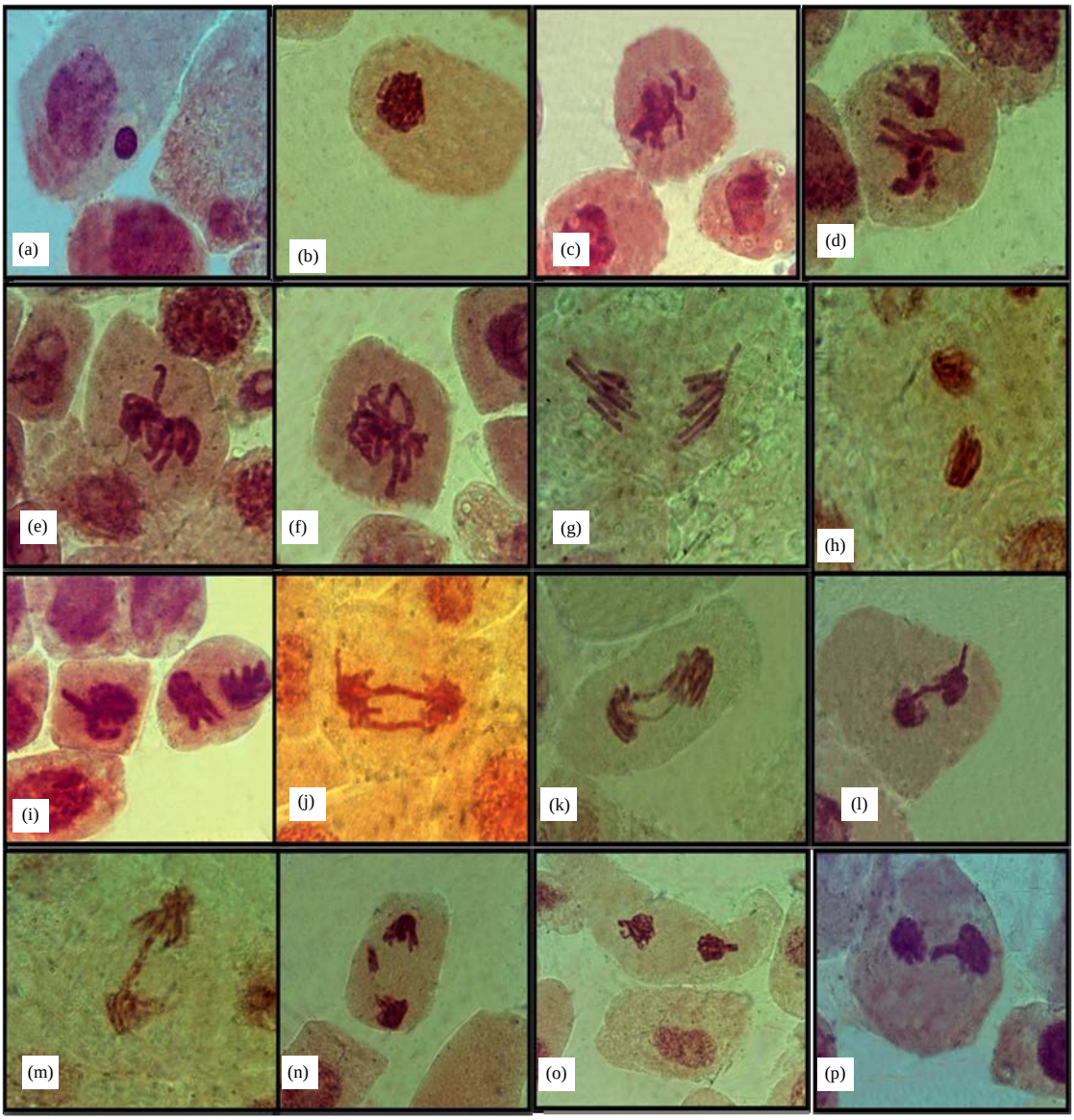


Fig. 1(a-p): Types of mitotic abnormalities induced by treatment of *Vicia faba* root tip cells with different antioxidants and their combinations with *T. harzianum* and *T. viride* (a) Micronucleus at interphase (SA-24 h), (b) Stickness at metaphase (($H_2O$+*T. viride*)-48 h), (c) Non-congression at metaphase ((SA+*T. harzianum*)-48 h), (d) Two groups at metaphase (CA-48 h), (e) Disturbed at metaphase ((SA+*T. viride*)-24 h), (f) Ring at metaphase (($H_2O$+*T. viride*)- 48 h), (g) Diagonal at anaphase ((CA+*T. harzianum*)-48 h), (H and I) Diagonal at telophase ((AA+*T. harzianum*)-24 h), (J) Two bridges at anaphase ((AA+*T. harzianum*)-24 h), (k-m) Bridge at telophase ((AA+*T. harzianum*)-24 h), (n) Laggard at telophase (($H_2O$+*T. harzianum*)-48 h), (o) Disturbed at telophase (AA-24 h) and (p) Late separation at telophase ((AA+*T. viride*)-24 h)

bridge, laggard, disturbed at anaphase stage and bridge, laggard, diagonal, late separation, disturbed at telophase as shown in Fig. 1.

Chromosomal abnormalities can be classified into three groups: 1st group which is known as mitotic abnormalities and include c-metaphase, polyploidy, laggard and multipolar division[32], these abnormalities are result of chemicals on spindle apparatus. 2nd group include chromosome breaks, stickiness and bridge. 3rd group include the formation of multinucleated cells and micronucleus.

The appearance of chromosomal abnormalities may be due to some factors that effect on cell division and from

these factors, using of herbicides, insecticides and fungicides[33]. Using pesticides also increases mitotic index and chromosomal aberrations[34]. Traffic pollution[35] and contamination with heavy metals[36] has the largest part in causing chromosomal abnormalities not only has a bad effect on plant but also for human who feed on these plants as it can have tumors as the result of this infection.

The highest percentage of abnormal mitosis was 43.53 and 37.24% at 24 h for 20 mM of (Aa+*T. harzianum*) and 3 mM of salicylic acid, respectively. On the other hand control sample 9.37%, while the highest abnormal mitosis percentage at 48 h was 64.93 and 43.13% for ($H_2O$+*T. viride*) and 20 mM citric acid.

Finally it is recommended to use bio-agents like antioxidants with bio-control fungi *T. harzianum* and *T. viride* and in future in replacement of chemical fungicides (unsafe and inexpensiveness). The lowest significant decreasing of abnormal mitosis was detected in combination of (citric acid with *T. viride* and citric acid with *T. harzianum*) compared with others.

## CONCLUSION

Biological control as *Trichoderma harzianum* or *Trichoderma viride* in addition to antioxidants is very important for decreasing pathogenicity of plant pathogens on plant. *Trichoderma* can be used safely as bio-control agents against pathogenic microbes.

## SIGNIFICANCE STATEMENTS

This study showed cytological aberrations in plants serve as an excellent monitoring system for the detection of mutagenicity. Although *Trichoderma* spp. are effective biocontrol agents against several fungal soil born plant pathogens.

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