



Journal of
Plant Sciences

ISSN 1816-4951



Academic
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Research Article

The Genotoxicity of Aphthitalite and Halite on *Vicia faba* Seedlings

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Abstract

Background and Objective: Synthetic dyes are commonly used as food additives because of their colouring properties uniformity, stability, and little cost. However, a lot of them become toxic after prolonged use, causing health problems and induced DNA damage and chromosomal anomalies in mammalian cells. Aphthitalite and Halite are components of synthetic dyes called Artificial Tamar Hindi are used as a famous beverage in Egypt. This is the first study on Aphthitalite and Halite. The objective of this research was to evaluate the side effects of Aphthitalite and Halite (AH) as one of the famous synthetic dyes are using in Egypt and reduce the side effects by using some natural material. **Material and Methods:** We used *Vicia faba* as a biological system to study the genotoxicity of AH. Green onion leaves, *Boswellia carterii*, and *Artemisia* spp. were the natural material used to avoid the chromosomal aberration induced by AH. **Results:** The results refer that AH (which is distinct as Aphthitalite and Halite by X-ray diffractions) reduces the rate of divisions and increases chromosomal anomalies such as laggard chromosome, micronuclei, double nuclei, bridge, and breaks. Onion leaves, *Boswellia serrata* and *Artemisia* reduce the side effects of AH concerning MI. All-natural materials reduce the chromosomal aberration induction by AH especially the onion leaves which significantly reduce the chromosomal aberrations followed by *Boswellia serrata*. About the shoot and root length, AH significantly increases in root length while in shoot length there are no significant differences compared with control. **Conclusion:** The results indicated that natural materials reduce side effects induction by AH.

Key words: Genotoxicity, Aphthitalite, Halite, halobacterium, chromosomal anomalies, *Vicia faba*, foods additives

Citation: Zedan, A.M.G., S.A. El-Shazly, M. Atef, A.A.Y. Mohamed and E.A.A. Ahmed *et al.*, 2022. The genotoxicity of Aphthitalite and Halite on *Vicia faba* seedlings. J. Plant Sci., 17: 27-32.

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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

Foods additives have been employed to improve the taste, tint, price of foods and constancy^{1,2}. A lot of people every day consume diverse food additives that have both advantages and disadvantages. Lately, progressive use of natural or synthetic food additives amplified the attention paid to their toxicity and benefit in food, particularly for the young³. Several colour additives are extracted from natural material such as carotene, while the others are synthetic, for example, tartrazine, indigotin, Allura red and carmoisine^{4,2}. Synthetic colours are widely used because of their stability, colour properties uniformity and low cost. Though, a lot of them turn out to be toxic after prolonged use. Synthetic dyes cause health problems, for example, anaemia, indigestion and allergic reactions such as asthma also, urticaria, pathological lesions in the brain, spleen, liver and kidney, Insulin hypersecretion, tumours and cancer paralysis^{5,6}. Also, colour additives induce DNA damage in insects, fungi, bacteria and mammalian cells *in vitro* and *in vivo*. They also cause chromosomal anomalies in mammalian cells^{7,8}.

One of the useful medical and aromatic plants is *Artemisia* sp. (wormwood) comprising about 500 species, many of them produce essential oils used in folk besides modern medicine, *Artemisia* sp. Also used in the pharmaceutical and cosmetics industry^{9,10}. wormwood are herbs used as feed additives and contains active ingredients for example, essential micronutrients, hormone-like agents, unknown factors, several of which are in charge of different biological activities as an analgesic, anti-inflammatory, anti-parasitic, anti-microbial, hypolipidemic, antioxidant, antinociceptive, hepato-protective, anti-malarial, antiulcerogenic, anti-diabetic, anti-leishmanial, anticonvulsant, anti-convulsant, anti-promastigote, anxiolytic and anti-depressant^{10,11}. Also, the oriental wormwood contains alkaloids, vitamins and minerals (vitamin A, B₁, B₂, C, Ca, P and Fe)¹².

Boswellia carterii has anti-cancer and anti-tumour properties. It showed inhibition of proliferation activity against, MCF-7, Bel-7402, K562, SMMC-7721 and HeLa cancer cell line^{13,14}.

The outer leaves of onion contain high amounts of flavonoids. It contains higher than 85% of superoxide and hydroxyl radicals which play an important role in free radical scavenging activities. Additionally, the onion outer leaves have higher antioxidant activity and higher reducing power in a linoleic acid system compared with potato, cabbage, spinach and crown daisy¹⁵.

This is the first study on Artificial Tamar Hindi powder as colour additives, which is classified as Aphthitalite and Halite plus the colour of halobacteria and this is the first study on Aphthitalite and Halite plus the colour of halobacteria and induction chromosomal aberration on *Vicia faba* as a biological system, also we used green leaves of onion as anti chromosomal aberrations and this is the first study in this field.

MATERIALS AND METHODS

Preparation of natural extracts: One gram of green onion leaves and *Boswellia carterii* were added to 99 mL distilled water to be 1% concentration, green onion leaves were ground in the mixer before being used. Five grams of *Artemisia* spp. were soaked for 6 hours in a hot water before they have used. ATH concentration was 0.1%.

Seeds treatment: *Vicia faba* seeds were obtained from the Sakha Agricultural Research Station, Egypt. This research project was conducted from 2018-2021. Seeds were soaked in distilled water for 24 hrs, it was left to grow for two days until the root becomes two cm long. *Vicia faba* seeds were distributed on the treatments as mentioned in Table 1, each treatment was fifty seeds. Random samples of developing roots were taken after 24 hrs of treatment for cytological analysis.

Cytological analysis: Root tips of *V. faba* seedlings for each replicate were cut then were fixed in carnoy's fixative solution which contains ethyl alcohol absolute mixed with glacial acetic acid with the ratio of 3:1. Root tips were fixed for 24 hrs., after that, they were kept in ethyl alcohol (70%) in the refrigerator until used for cytological analysis. Cytological studies were carried out using 2% aceto-carmine stain¹⁶. Cells were examined under a light microscope for numbers, mitotic index and types of abnormalities with the examination of at least 3000 cells in each treatment (1000 cell/replicate).

Table 1: Code and concentrations of treatments

Treatments	Concentration (%)
Negative control (distilled water)	
Positive control (artificial tamar hindi ATH)	0.1
Green onion leaves (GL)	1
ATH+green onion leaves (ATHGL)	0.1+1
<i>Boswellia carterii</i> (Bc)	0.1
ATH+ <i>Boswellia carterii</i> (ATHBc)	0.1+0.1
<i>Artemisia</i> spp. (As)	5
ATH+ <i>Artemisia</i> spp. (ATHAs)	0.1+5

Mitotic Index (MI) and the percentage of abnormal cells were measured using the following formulas¹⁷:

$$\text{Mitotic Index (MI)} = \frac{\text{Total dividing cells}}{\text{Total dividing and non dividing cells}} \times 100$$

$$\text{Abnormal cells (\%)} = \frac{\text{Total abnormal cells}}{\text{Total dividing cells}} \times 100$$

RESULTS

X-ray diffraction analysis: The X-ray diffraction picture for colour additives of Artificial Tamer Hindi powder (ATH) refers that it consists of Aphthitalite (61.5%) and Halite (sodium chloride 38.5%) as shown in Fig. 1.

The length of shoot and root: Table 2 illustrated the effect of ATH on the length of shoot and root for *Vicia faba* seedlings, concerning root length the treatment ATHBs gave the highest value (9.20 cm) whereas As gave the lowest value (2.92 cm) followed by ATHAs (3.42 cm). About shoot length, there were significant differences between treatments, GL recorded the highest value (5.05 cm) while control negative and positive recorded the lowest value (2.28 and 2.32 cm, respectively).

Cytogenetic analysis: There were significant differences between the treatments for the rate of mitosis index and chromosomal aberrations. ATHBc recorded the highest rate of mitotic index in comparison to the other treatments and negative control, this agrees with the results belonging to the

root length. ATH significantly recorded the lowest in the mitotic index. This indicated that ATH inhibits the cell cycle also increases the chromosomal aberrations as shown in Table 3. From the results we concluded that green onion leaves reduce the side effects of ATH, also the other treatments significantly reduce the side effect of ATH. The data in Fig. 2a-d showed a laggard chromosome, whereas Fig. 2e-f showed micronuclei. Also, Fig. 2g showed double nuclei and Fig. 2h-i showed bridge and breaks, respectively. With the mitotic index, ATHBs recorded the highest rate of mitotic index in comparison to the other treatments and negative control, this agrees with the root length, it was followed by ATHGL, whereas ATH was the lowest in the mitotic index (Table 3).

Concerning the chromosomal anomalies, there were significant differences between the treatments. ATH recorded the highest ratio in the chromosomal aberrations compared with the other treatments. Negative control and ATHGL recorded the lowest rate in chromosomal anomalies (Fig. 2).

Table 2: Effect of artificial Tamer Hindi on the length of shoot and root of *Vicia faba* seedlings

Treatments	Root length (Means±SD)	Shoot length (Means±SD)
Control	4.65±1.43 ^d	2.28±0.92 ^c
ATH	5.68±2.04 ^{bc}	2.32±1.07 ^c
GL	6.23±2.12 ^{bc}	5.05±2.79 ^a
ATHGL	5.52±1.81 ^c	3.78±2.08 ^b
BC	6.47±2.19 ^b	3.96±2.59 ^b
ATHs	9.20±2.78 ^a	3.84±2.14 ^b
As	2.92±0.74 ^e	3.56±1.80 ^b
ATHs	3.42±0.66 ^e	3.23±1.63 ^b
Significant	0.00	0.00

ATH: Artificial Tamar Hindi, GL: Green onion leaves, ATHGL: ATH+green onion leaves, Bc: *Boswellia carterii*, ATHBc: ATH+ *Boswellia carterii*, As: *Artemisia* spp, ATHAs: ATH + *Artemisia* spp and values with different letters in each column showed a significant difference

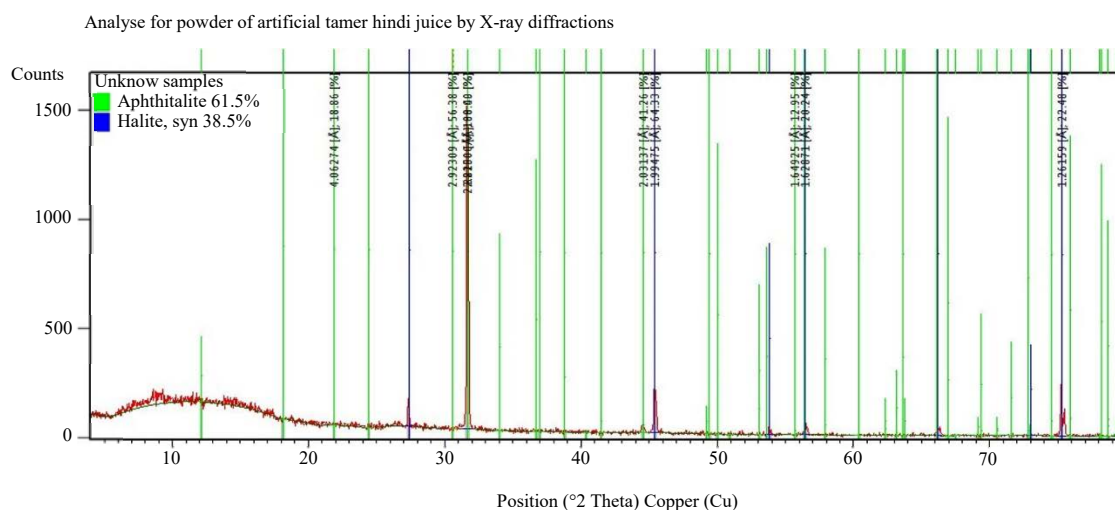


Fig. 1: X-ray diffractions picture for artificial Tamer Hindi

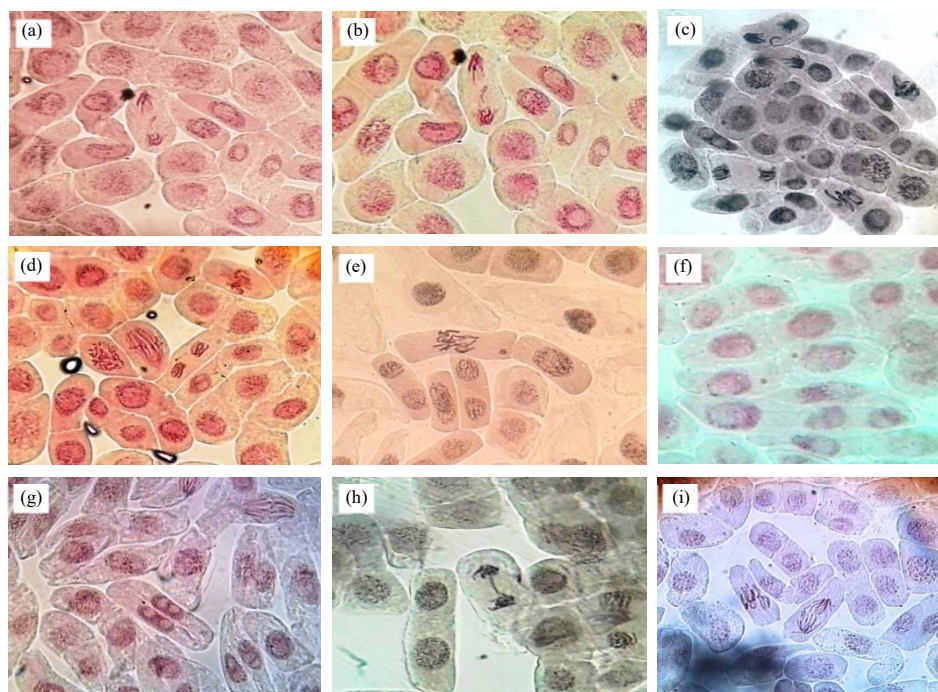


Fig. 2(a-i): Types of abnormalities observed in *V. faba* root tips treated with artificial Tamer Hindi and other combined natural materials, (a,b,c and d) Laggard chromosome, (e and f) Micronuclei, (g) Double nuclei, (h) Bridge and (i) Breaks

Table 3: Mitotic index and chromosomal aberrations of *V. faba* seedlings treated with artificial tamarind only or combination with other natural material

Treatments	No. of examined cells	No. of dividing cells	No. of abnormal cells	MI (%)	AB (%)
Control	3051	195	2	6.38±1.71 ^{ab}	0.92±0.82 ^c
ATH	3846	121	40	3.23±1.18 ^c	34.60±8.92 ^a
GL	3107	227	14	7.32±0.88 ^a	6.19±1.35 ^b
ATHGL	2794	192	0	6.84±0.35 ^{ab}	0.00±0.00 ^c
Bs	3502	170	12	4.84±0.89 ^{bc}	6.91±6.01 ^{bc}
ATHBc	3619	300	9	8.27±1.42 ^a	2.71±2.82 ^{bc}
As	3581	274	40	7.61±1.51 ^a	11.45±7.48 ^b
ATHAs	3366	209	17	6.22±1.41 ^{ab}	8.21±7.12 ^{bc}
Significant				0.003	0.000

ATH: Artificial tamar hindi, GL: Green onion leaves, ATHGL: ATH+green onion leaves, Bc: *Boswellia carterii*, ATHBc: ATH+*Boswellia carterii*, As: *Artemisia* spp, ATHAs: ATH + *Artemisia* spp and values with different letters in each column showed a significant difference

DISCUSSION

X-ray diffraction has long been considered a necessary crystallographic technique based on Bragg's law¹⁸. To determine the ATH powder, X-ray diffraction was the best method. ATH powder contains Apththalite and Halite. The pink colour of ATH comes from halite crystals. The pink colour is coming from the Halobacterium organism which lives in the concentrated brine¹⁹. The halophilic bacteria have been noted for their pink colour or bright red²⁰. The mineral Apththalite classified as a non-hydrated sulfate of sodium and potassium is one of the scarcer of the native soluble sulfates. The structure is variable but has been generally

classified as $(K, Na)_2SO_4$, with $K_2O : Na_2O = 3:1$. $(K_3 Na_1 O_8 S_2)^{21}$. Apththalite is mixed with an industrial clinker and added low alkali and sulfate substance to regulate the contents of alkali sulfates in clinker²². These colour additives were used in the preparation of Artificial Tamer Hindi as a famous drink in Egypt. This is the first studying for the effect of Apththalite and Halite with the pink colour of Halobacterium on the chromosomal aberration of *Vicia faba* as a biological system. This study is the first step for more studies on higher biological systems such as rats or cell lines.

The results in Table 2 showed the effect of ATH on root and shoot length, Also the other treatments and their role in the growth parameter. The negative control showed a lower

value about ATH. ATHBc treatment gave the highest value in root length whereas GL recorded the highest value in shoot length. There are no references about the improvement of the shoot length by green onion leaves, the aqueous extracts of fresh green onion leaves which are characterized by FTIR spectral analysis show phenols, alkynes, aldehyde and alkyl halides compounds²³.

These results agree with reports about GL components and the role of antioxidant activities, green onion leaf contains quercetin which is considered as flavonoids²⁴. Quercetin, a polyphenol resulting from plants, has a broad range of biological activities including anti-inflammatory, antiviral activities and anti-carcinogenic, in addition to attenuating lipid peroxidation, capillary permeability, also platelet aggregation²⁵. Quercetin has a defensive character in the abatement of dimethoate-induced cytotoxicity and genotoxicity in the allium sativum-meristematic cells that resides on its antioxidant properties²⁶.

The extract of *Boswellia serrata* showed a significant protective property in opposition to mutagenicity induced by mutagen in *S. typhimurium* TA100 and TA98 strains²⁷. Boswellic acid inhibited the induction of micronuclei creation by clastogen. It also has a significant defensive effect against the cyclophosphamide-induced incidence of chromosomal aberrations²⁸. We noticed that GL, Bs as have rated in chromosomal aberrations alone, these aberrations reduce when combining with ATH *Artemisia* spp.

CONCLUSION

Our work is important to the reader because it belongs to the spread of food additives. For the first time, the x-ray diffraction proved that one of the most spread food additives consists of Aphthitalite and Halite that caused chromosomal aberrations in *Vicia faba* seedlings. The use of natural materials reduces the side effects of these artificial food additives.

SIGNIFICANCE STATEMENT

This study discovers the structure of the most common artificial food additives and the role of some natural extracts that can be beneficial for alleviating the side effects of artificial food additives. This study will help the researcher to uncover the critical areas of Aphthitalite and Halite that many researchers were not able to explore. Thus, a new theory on the role of this material that already natural exists may be arrived at using it in another study that proves it as a carcinogen material.

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