Influence of Methyl *tert*-Butyl Ether (MTBE) on white *Corn (Zea mays* L.) Plant Growth

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Abstract: This work was designed to investigate the phyototoxicity of methyl *tert*-butyl Ether (MTBE) to metabolic activity of white corn (*Zea mays* L.) plants. The four-week-old potted plants were subjected to four weekly doses (50 ml of MTBE) at different concentrations (0.5, 1.0, 5.0, 10 and 15 %). Growth parameters indicated significant ($p \leq 0.05$) inhibition only at the high concentrations (0.5 and 1.0 %) of MTBE. The leaf area and chlorophyll-A contents decreased as the concentration of MTBE increased. A marked increase of lipid peroxidase activity was recorded at the different MTBE concentration, while a slight decrease of catalase activity was recorded at the same MTBE concentration. The impaired growth and anabolic activities in white corn plant resulted from the oxidative stress of MTBE.

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1. Introduction

Since 1992, methyl *tert*-butyl ether (MTBE) has been added to gasoline, instead of lead, worldwide as oxygenate in order to enhance combustion efficiency to lower carbon monoxide emission to reduce air pollution . It is the most commonly used as fuel oxygenate because of its low cost , high octane level and case of blending with gasoline (**Johnson, 2000**). The U.S. EPA has classified MTBE as possible human carcinogen (**Squillace, 1996**) .

Methyl *tert*-butyl ether (MTBE) is a chemical compound with molecular formula $C_5H_{12}O_5$, it is the second high produced industrial chemical in the USA and frequent ground water pollutant . MTBE is quit persistent to biotic and abiotic decomposition.

The cause of MTBE leaking into the environment is mainly attributed to gasoline spills and leaks from pipelines, underground and above ground storage tanks, and transport accidents (**An, 2002**). As a result, increased soil contamination by MTBE can lead to inhibition of soil microflora which alter soil health and fertility (**Mehler, 2001**). Toxicity of MTBE to aquatic plants has been intensively studied (**Werner, 2001**). Yet, few works has been conducted on the toxicity of MTBE to terrestrial plants (**Nellessen, 1993**). Therefore, the main objective of this study was to assess its effect on seed germination, plant growth, photosynthesis and some enzyme activity in white corn (*Zea mays* L. cv. 310) plants.

2. Material and Methods

Plant material

The experimental plant used in the current study was pure strain of *white corn (Zea mays* L. cv 310). Certified seeds were provided by the Agricultural Research Center in Giza , Egypt .

Methyl tert-butyl ether (MTBE)

The high grade chemical MTBE was obtained from ARAMCO Jeddah, Saudi Arabia.

Growth experiment

The experiment was conducted in the Botanical garden of the Faculty of Science, Taif University, Saudi Arabia in the Spring of 2011. For plantation, 30 plastic pots were filled with homogenous pre-sieved garden soil (Sandy loam). Seeds were soaked in the pot soil about 3 cm deep. All pots were watered up to saturation, then kept in the open garden and irrigated regularly to field capacity until MTBE treatments.

MTBE treatments

After two weeks from soaking, the planted pots were randomly subdivided into six equal groups (3 pots each). One group was treated with pure water and sampled as control. The other five groups were subjected to four weekly doses (50 ml of MTBE) At different concentrations (0.5, 1.0, 5.0, 10 and 15 %) added to the soil with 50 ml of ½ Hoagland nutrient solution .

Sampling and measurements

Growth vigor

After four weeks from soaking , vegetative growth parameters (shoot and root lengths, their fresh and dry weights and the area of third foliage leaf) were recorded **.**

Leaf area: The area of the third foliage leaf was determined using the formula:

A = L × W × 0.75;

where L = the leaf length , W = the leaf width , 0.75 is the factor of recalculation for maize leaf (**Montgomery, 1970; Whigham *et al*., 1974; Pearce *et al*., 1975; Aliu *et al*., 2008**).

Chemical analysis

Photosynthetic pigments

By the end of the sixth week, chlorophyll-A, Chlorophyll-B, and carotenoids were estimated (ugml^{-1}) in the third fresh foliage leaf. One gram of fresh leaf tissue was extracted by grinding in 10 ml of 80% acetone. The mixture was then centrifuged for 5 min. at 3000 rpm. The supernatant was used for spectrophotometric determination according to the method of **Lichtenlhaler and Wellborn (1983)**.

Antioxidant and oxidative enzymes

Catalase Enzyme

Catalase activity was measured according to the method of **Chen and Moely (1992)**. Extracts were prepared 4 weeks after imposing the MTBE in the nutrient solution. Plant tissues were homogenized with 0.1 M sodium phosphate buffer (pH = 6.8) in a chilled mortar and pestle. The homogenate was centrifuged at 14000 rpm for 20 min. The obtained supernatant was used for the determination of enzyme activity. The whole extraction procedure was carried out at 4° C.

Lipid peroxidase enzyme

The thiobarbituric acid reactive substance (TBARS) levels as index of malondi-aldehyde (MDA) production were measured by the method described by **Ohkawa *et al.* (1979)**. MDA, an end product of lipid peroxidation reacts with TBA-TCA complex to form a colored complex at high temperature exhibiting an absorption maximum at 535 nm. Plant tissue (0.5g) was homogenized using a potter–Elvejham homogenizer with 3 ml of 0.1 M of ice-cold phosphate buffer (pH = 7.4) . The homogenate was centrifuged for 10 min at 12000 rpm. A volume of 100 µl from the supernatant was mixed with 100 µl of normal saline and 400 µl of TBA-TCA mixture, all were incubated in a boiling water bath for 10 min., then cooled to room temperature. After centrifugation at 1200 rpm for 10 min, 100 µl from the supernatant was mixed with 100 µl of 0.7 % TBA in a cuvette and the absorbance was read at 535 nm. The concentration of MDA was calculated using a standard curve from 1,1,3,3,-tetraethoxypropane and expressed as µmolg^{-1}.

Statistical analysis

Growth parameters were statistically analyzed using multiple comparison procedure at $p \leq 0.05$ using t-test and mean separation by least significant difference (LSD) (**Steel and Torrie, 1980**).

3. Results

In response to the investigated MTBE concentrations, the results of growth parameters of white corn (Table 1) indicated non-significant ($p \leq 0.05$) effects on all growth parameters at low concentrations (0.5 and 1.0 %) of MTBE; while, the high concentrations (5.0, 10, and 15%) significantly inhibited the stem and root lengths of the plant, the stem and root fresh weights, stem and root dry weights. The severity of inhibition increased as the concentration of MTBE increased above 5%.

The leaf contents of chlorophyll a and total chlorophyll (Table 2 & Figure 1) were reduced by all the applied MTBE concentrations; while, the contents of chlorophyll b and carotenoids as well as the ratio of chl. a/chl. b were almost unchanged. The area of the third foliage leaf (Figure 2) were reduced as the concentration of MTBE increased. The activity of catalase enzyme in the tissues of white corn seedling (Figure 3) was inhibited in response to the highest concentration (15%) of MTBE. However, the activity of lipid peroxidase enzyme showed slight increases in response to the low concentration (0.5, 1.0 and 5%) of MTBE and remarkable enzyme activity in response to the high concentrations (10 and 15%) of MTBE.

4. Discussion

Among the responses of vascular plants to organic chemicals are growth parameters (**Nellssen and Fletche, 1993**). In the current investigation, a generalized inhibitory influence of MTBE on white corn plants is reported. Earlier laboratory studies reported negative growth responses of certain algae to MTBE (**Rousch and Sommerfeld, 1998**). Further studies (**Cape *et al.,* 2003**) reported on significant effects of volatile organic compounds (VOC) including MTBE on leaf water content and photosynthetic capacity of some plant species. Moreover, severe toxic symptoms were detected in weeping willow (*Salix babylonica* L.) after 120 hrs of exposure to MTBE as shown as significant reduction (35%) in transpiration. However, leaf chlorosis was not detected for the whole duration of the test (**Yu and Gu, 2006**).

Holding fairly to our results, **Youn-Joo *et al.* (2002)** reported on a reduction in seed germination, shoot and root growth of oats, sweet corn, wheat and lettuce subjected to different MTBE concentrations in the soil. Root growth, flower and pod development were found to be more sensitive to MTBE treatments,

while stem growth and photosynthetic pigment contents were more persistent to the toxicity of MTBE in common bean plants (**Beltagi, 2007**).

MTBE is quite persistent to abiotic decomposition, in other words, the natural attenuation of MTBE in aquifers is slow and, in some cases, undetectable with half-life of at least two years (**Fayolle *et al.* 2001**). Thus, MTBE is highly water soluble and is readily absorbed by plants. The obtained data of this investigation showed reductions in both shoot and root growth and reduced leaf area as well. These observations assume that MTBE might be absorbed, accumulated and transported with the plant parts from root to shoot. The decrease in the leaf area and the leaf content of chlorophyll a suggest photosynthesis as a target for the phytotoxicity of MTBE.

All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

A variety of environmental stresses including soil salinity, drought, extremes of temperatures, and heavy metals are known to cause oxidative damage to plants either directly or indirectly by triggering an increased production of reactive oxygen species (ROS) (**Shalini Verma and Dubey, 2003**). To combat the oxidative damage, plants have the antioxidant defense system comprising of enzymes such as catalase, peroxidase, superoxide dismutase and the non-enzymic constituents as well which remove, neutralize and scavenge the ROS. A decline in catalase activity in response to MTBE concentration was observed in our study which suggest a possible delay in the removal of H_2O_2 and toxic peroxides mediated by catalase and in turn an enhancement in the free radical mediated lipid peroxidation under MTBE toxicity. The decline in catalase activity is regarded as a general response to many stresses (**Herbinger *et al.*, 2002; Bakalova *et al.*, 2004; Jung, 2004; Pan *et al.*, 2006; Gunes *et al.*, 2008; Liu *et al.*, 2008**). The reduction in catalase activity could be attributed to the inhibition of enzyme synthesis through the change in the assembly of enzyme subunits under MTBE stressful condition. On the other hand, our results indicated an enhancement in the activity of lipid peroxidase, suggesting a significant role for this enzyme as an intrinsic defense tool to resist MTBE oxidative damage in white corn plants (**Verma and Dubey, 2003**). The enhanced antioxidative scavenging mechanism of lipid peroxidase activity might be considered as an important mechanism to cope with MTBE oxidative stress as reported by **McKersie *et al.* (1999),), Das & Csiszar *et al.* (2005) Uprety (2006) and Gunes *et al.* (2008** for peroxidase activity under drought stress.

In conclusion, MTBE causes oxidative stress in white corn plants and catalase and peroxidase enzymes appear to have a pivotal role in combating oxidative stress in white corn plants. The increased activity of the involved enzymes in removing reactive oxygen species, like lipid peroxidase, resulted from the stimulation of gene expression to alleviate the adverse effects of oxidative stress caused by MTBE.

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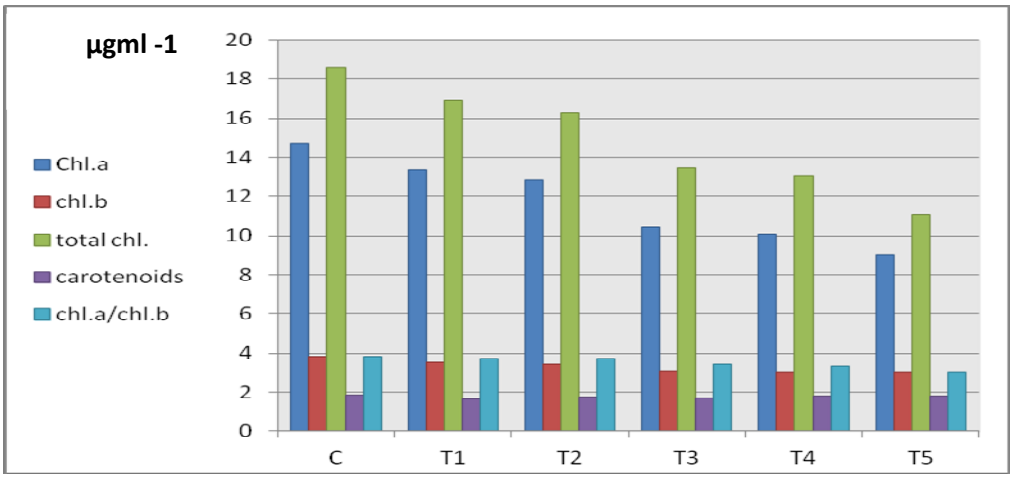
Table 1. Mean growth parameters of white corn (*Zea mays* L. cv. 310) plants subjected to 0.0, 0.5, 1.0, 5.0, 10 and 15 % MTBE concentrations (each value is a mean ±SD of 3 replicates).

MTBE conc. (%)	Stem fresh weight (gm)	Root fresh weight (gm)	Stem length (cm)	Root length (cm)	Stem dry weight (gm)	Root dry weight (gm)
0.0	6.08±0.005	4.18±0.045	30.81±0.76	26.32±0.080	0.47±0.015	**0.49±0.020**
0.5	5.77±0.020	3.87±0.030	29.43±0.62	23.55±0.509	0.44±0.032	**0.46±0.036**
1.0	6.47±0.040	3.60±0.064	25.20±0.06	21.17±0.282	0.42±0.020	**0.36±0.020**
5.0	2.35±0.018	1.41±0.015	18.65±0.07	20.22±0.115	0.21±0.015	**0.35±0.025**
10	2.27±0.015	0.81±0.035	18.13±0.04	19.23±0.080	0.17±0.010	**0.31±0.015**
15	1.33±0.035	0.47±0.025	15.27±0.05	15.12±0.068	0.13±0.015	**0.26±0.025**

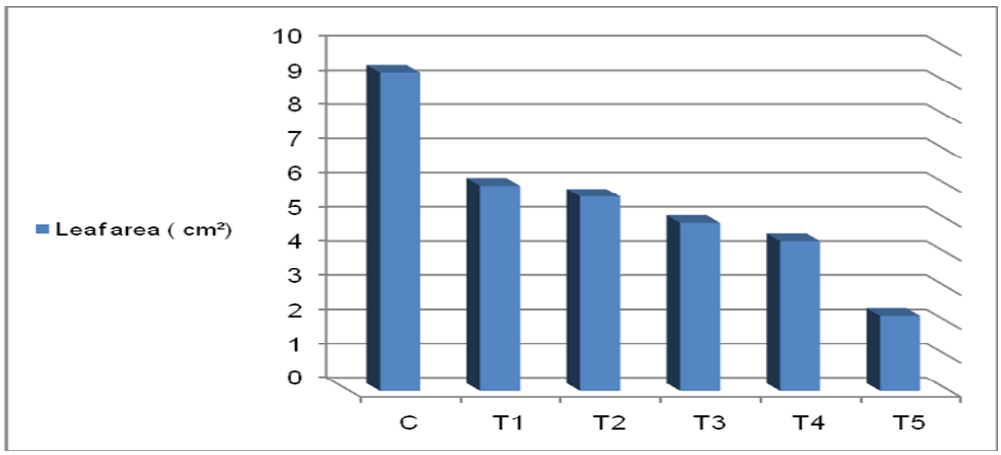
Table 2. Chlorophyll a (chl. a), chlorophyll b (chl. b), total chlorophyll (total chl.), carotenoids and chlorophyll a/chlorophyll b (chl. a/chl. b) leaf contents in white corn (*Zea mays* L. cv. 310) subjected to 0.0, 0.5, 1.0, 5.0, 10 and 15 % MTBE concentrations.

MTBE concentrations (%)	Chl.a (μgml^{-1})	Chl. b (μgml^{-1})	Total Chl. (μgml^{-1})	Carotenoids (μgml^{-1})	Chl. a/chl. B
0.0	14.73	3.85	18.58	1.83	**3.82**
0.5	13.34	3.54	16.88	1.64	**3.73**
1.0	12.83	3.45	16.26	1.73	**3.71**
5.0	10.43	3.03	13.44	1.70	**3.41**
10	10.03	3.01	13.04	1.78	**3.33**
15	09.03	3.00	11.04	1.80	**3.00**



MTBE treatments (%)

Figure 1. Chlorophyll a (chl.a), chlorophyll b (chl. b), total chlorophyll (total), carotenoids and chlorophyll a/chlorophyll b (chl.a/chl.b) leaf contents in white corn (*Zea mays* L. cv. 310) subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15 %) MTBE concentrations.



MTBE treatments (%)

Figure 2. The area of the third foliage leaf of white corn (*Zea mays* L. cv. 130) plants subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15 %) MTBE concentrations.

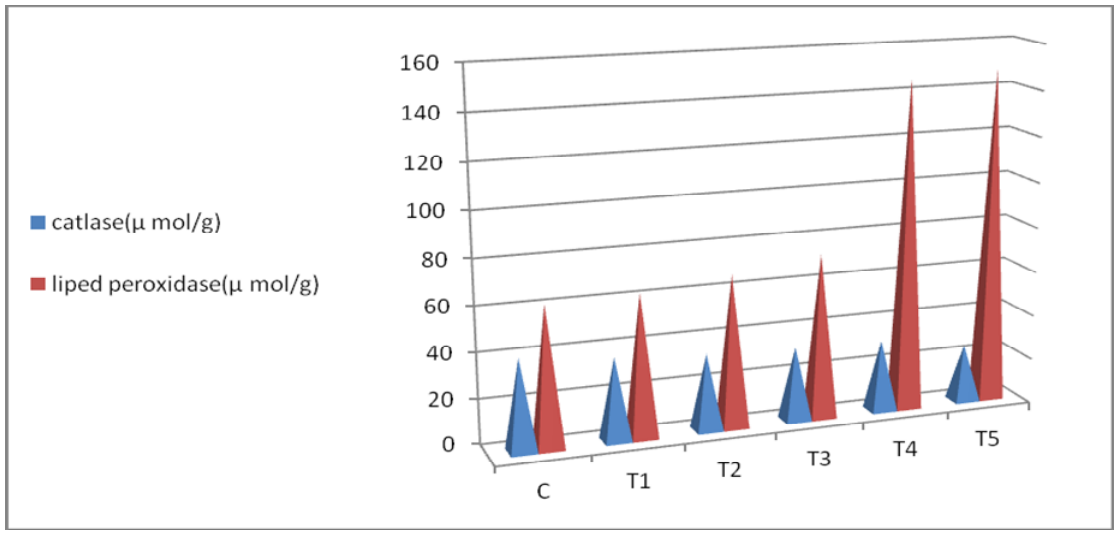


Figure 3. Activity of catalase and lipid peroxidase in leaves of white corn (*Zea mays* L. cv. 310) subjected to C, T1, T2, T3, T4 and T5 (0, 0. 5, 1.0, 5.0, 10 and 15

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