

Effect of Methyl *tert*-Butyl Ether on Growth and Phosphoenolpyruvate Carboxylase activity of *Zea mays*

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Abstract: This work was designed to investigate the phytotoxicity effect of Methyl *tert*-Butyl Ether (MTBE) to maize plants. One week old seedlings were exposed continuously for 7 days to 5 different MTBE concentrations in the nutrient medium (0, 20, 40, 60 and 80 ml/L.). All growth parameters tested (pigment content , leaf area , root and shoot growth) were sensitive to MTBE, shoot length was more reduced in MTBE contaminated solution than was root length, which indicated that MTBE might be transported within the plant from the roots to the shoots.. The reduction in pigment content is related mainly to the reduction of Chl. a rather than Chl. b. A marked increase of PEP carboxylase activity was recorded only at concentrations 20 and 40 ml/L.MTBE, whereas the enzyme activity was strongly inhibited in plants subjected to the highest concentration (80ml/L. MTBE).

Keywords: Methyl *tert*-Butyl Ether, Phytoremediation, Phosphoenolpyruvate carboxylase, Hydrophobicity.

INTRODUCTION

Plant uptake of organic pollutants is governed by the chemical and physical properties of the pollutant, the environmental conditions and the plant species

Phytoremediation of organic pollutants primarily occurs by three mechanisms: (1) Phytoextraction: the uptake and translocation of pollutants from soil into plant tissue; (2) Phytodegradation: the breakdown of organic pollutants either within plant tissue or in the microbe-rich rhizosphere; (3) Phytovolatilization: the transfer of the pollutant to air via the plant transpiration stream. Methyl *tert*-Butyl Ether (MTBE) is a chemical compound with molecular formula $C_5H_{12}O$. Is the second most highly produced industrial chemical in the US and a frequent groundwater pollutant .At the same time, MTBE is quit persistent to biotic and abiotic decomposition. The U.S. Environmental Protection Agency has classified MTBE as a potential human carcinogen (Mennear, 1997). Animal exposure studies have indicated that MTBE is a carcinogen and the influence of MTBE on freshwater organisms; including algae, bacteria, fish, amphibians, and invertebrates has been reported (Kampbell *et al.*, 2001; Rousch and Summerfield, 1998; Gupta and Lin, 1995; Werner *et al.*, 2001). Several reports have also appeared regarding the toxicity of MTBE to aquatic organisms, but little information is available about the toxicity of MTBE to terrestrial organisms such as plants. MTBE is biodegradable to CO_2 and water under aerobic conditions with the correct bacteria. However, the natural occurrence of these bacteria with the ability to break ether bonds is not high, and it appears that most strains of MTBE-oxidizing bacteria are slow-growing bacteria with low biomass production per unit MTBE oxidized (Fischer *et al.*, 2005). Plant uptake and transpiration of organic compounds is found to depend upon the contaminants hydrophobicity, quantified by its octanol-water coefficient K_{ow} . The process is most effective for pollutants with log K_{ow} in the optimum range of 0.5 – 3.0 (Burken and Schnoor, 1998). Since the log K_{ow} for MTBE is 1.24 (Chemfate, 1994), which is in the range that is readily transpired by plants, phytoremediation may be a feasible remedial technique for MTBE. In addition, MTBE may be metabolized within plants to benign lignin that contain ether linkages (Ramsden, 2000). Quantitative data on MTBE uptake and transpiration is required for design of engineered MTBE phytoremediation systems.

This work was designed to investigate the phytotoxicity effect of Methyl *tert*-Butyl Ether (MTBE) to maize plants.

MATERIALS AND METHODS

Maize (*Zea mays*) grains were washed twice with distilled water, followed by soaking in 0.01M $HgCl_2$ for 2 min. then extensive washing in dist. Water several times. Five-day old seedlings were grown hydroponically in growing units; each consists of 5 tubes of diameter 5cm and length 70cm. About 20 holes

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were made in each of the plastic tubes, and the seedlings were put each in a small Eppendorf (after cutting about 4mm of the tip). The Eppendorfs were then fixed in the holes. Seedlings were irrigated with a complete strength Hoagland solution. An air pump (200ml/min.) was put in the inlet of the tube to provide good circulation and aeration of the solution. The pH was kept within the range of 6.0 to 6.5. The experimental design consisted of 5 different MTBE concentrations (0, 20, 40, 60, and 80ml/L) added to the nutrient solution. The solutions were made using dist. Water, and the volume of the solutions was maintained by adding dist. Water. The growth units were kept in a controlled chamber with temperature (25°C), under a 13 hr. light/11hr. dark cycle and a regime of light intensity of 200 Wm⁻² at plant height.

Sampling and Measurements:

After one week of imposing MTBE (2-weeks-old seedlings), growth parameters (chlorophyll content, shoot and root lengths, fresh and dry weights and leaf area) were recorded. All parameters were statistically analyzed by multiple comparison procedure at 5% significance level (P = 0.05) using *t*-test. Measurements were made in triplicates.

Chlorophyll Determination:

About 1.0g of fresh leaf tissue was ground using 10 ml of 80 % acetone. The mixture was then centrifuged for 15 min. at 3000rpm. The supernatant was used to determine Chl. a, Chl. b and carotenoides by reading the absorbance at 663, 646 and 470 nm against acetone.

Leaf area:

Leaves were detached, blotted gently, drawn on paper then the area was measured with a leaf area- meter connected with a computer (portable Area Model LI-3000). Protein content was determined by the method of Bradford (1976) with BSA as the standard protein.

Extraction of PEP Carboxylase:

Extracts were prepared 7 days after imposing the MTBE in the nutrient medium. Approximately 1.0g of leaf tissue was homogenized at 4°C with purified sand and 10 ml of homogenization buffer, which has the following composition: 0.1M Tris HCl (pH 7.8), 0.5mM EDTA, 1mM MgSO₄ and 1mM DTE (freshly prepared). The method is slightly modified from that described by Foyer *et al.* (1994) to suit the quantities of the enzyme present in the leaves investigated. The extract was centrifuged in a Beckman-centrifuge (model 2-21) for 25 min. at 16,000 rpm. Approximately 0.3g of soluble polyvinyl pyrrolidone (PVP) was added to 3ml of the supernatant and mixed vigorously with a stirrer for 3 sec., and then the mixture was centrifuged at 4°C for 10 min. at 8000 rpm. The clear supernatant of the second centrifugation was used as the source of the enzyme.

Assay of PEP Case:

Activity of PEPCase was determined spectrophotometrically as described by Blanke *et al.* (1986) at 340nm by coupling the reaction to the oxidation of NADH in the presence of malate dehydrogenase (MDH). The standard assay medium contained the enzyme extract, 10 units of MDH, 0.1mM NADH, 2.5mM MgSO₄ and 5mM NaHCO₃ in a total volume of 2.95ml 50mM Tricine buffer (pH 8.8). The reaction was started by the addition of 50µl of PEP at 2.2mM final concentration. The rate of oxidation of NADH was measured 15 sec. after the addition of PEP over 3 min. The reaction was observed using the visual display of the spectrophotometer (Cecil CE 7200 split-beam spectrophotometer) to confirm the adequate mixing of the cuvette contents and that NADH oxidation caused by the reaction was linear. Assays were done in triplicate. Results were based on the soluble protein content.

RESULTS AND DISCUSSIONS

Results:

Growth parameters of *Zea mays* seedlings grown under different levels of MTBE are summarized in Table (1). A direct relationship was found between the severity of the response and increasing MTBE concentration in the nutrient medium. Shoots were generally more susceptible to MTBE than roots. The highest concentration of MTBE (80ml/L.) resulted in a significant reduction in shoot length which corresponds to about 49% of the control plants. MTBE treatment resulted in a significant reduction in root growth (length, fresh weight and dry weight). Inhibition of leaf elongation was one of the primary effects of MTBE. Leaf area as a growth parameter was highly affected in seedlings grown under 60 and 80ml/L. MTBE. After one week of exposure leaf area decreased by about 20% and 31% respectively.

Table 1: Mean growth parameters of *Zea mays* plants after one week of treatment with different MTBE concentrations in the nutrient medium.

Treatment	Leaf area (cm ²)	Shoot length (cm)	Shoot F.wt. (g)	Shoot D.wt. (g)	Root length (cm)	Root F.wt. (g)	Root D.wt. (g)
C	17.71	16.4	0.171	0.021	9.80	0.152	0.018
T1	15.80	14.09	0.118	0.019	6.51	0.129	0.015
T2	14.61	12.10	0.109	0.013	5.41	0.120	0.015
T3	14.20	10.80	0.019	0.011	4.82	0.087	0.016
T4	12.11	8.31	0.013	0.009	4.31	0.062	0.012
LSD (P = 0.05)	3.132	4.220	0.281	0.023	2.231	0.244	0.011

C = control plants, T1, T2, T3 and T4 plants treated with 20, 40, 60 and 80ml/L. MTBE

Table 2: Pigment content of *Zea mays* leaves after one week of treatment with different MTBE concentrations in the nutrient medium.

Treatment	Pigment content (mg/g.f.wt.)				
	Chl. a	Chl. b	Carotenoides	Total pigments	LSD (P = 0.05)
C	14.73	3.85	1.83	20.41	0.886
T1	13.04	3.54	1.64	18.22	0.541
T2	12.83	3.12	1.73	17.68	0.316
T3	10.94	3.05	1.70	15.69	0.283
T4	10.03	3.01	1.73	14.77	0.273

C = control plants, T1, T2, T3 and T4 plants treated with 20, 40, 60 and 80ml/L. MTBE.

Generally, Chl. a, Chl. b and total pigments decreased with increasing MTBE concentration, whereas Carotenoides remained more or less constant. The reduction in pigment content is related mainly to the reduction of Chl. a rather than Chl. b (Table 2).

On the other hand PEP carboxylase activity increased markedly as a function of increasing MTBE concentration in the nutrient medium. However, imposing the highest MTBE concentrations (60 and 80ml/L), caused a significant retardation of the enzyme activity (Fig.1)

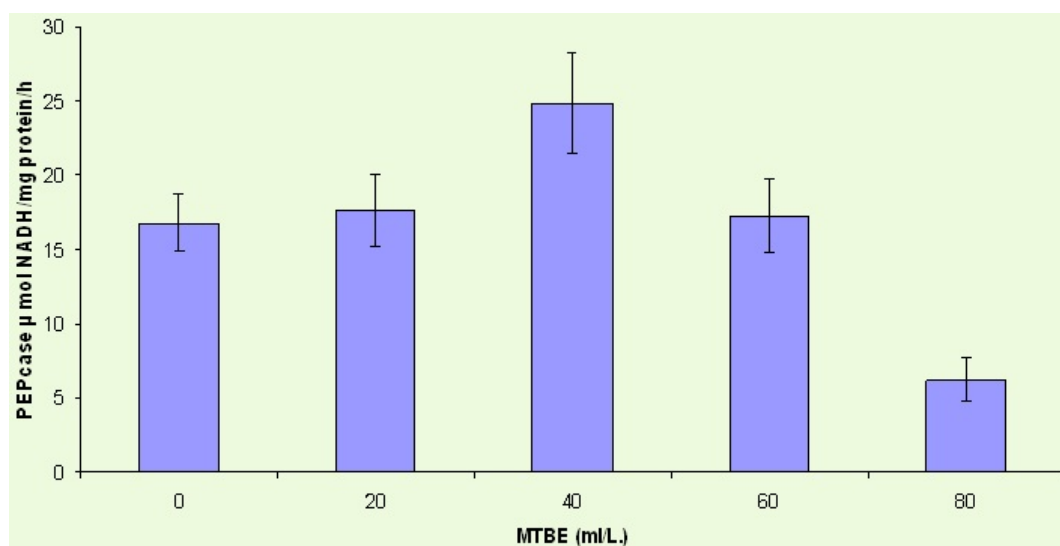


Fig. 1: Activity of PEP Case in shoots of *Zea mays* seedlings subjected to different concentrations of MTBE in the nutrient medium.

The initial (0.0 time) PEPCase activity in leaves of maize seedlings was 14.8µmol NADH/gf.wt./hr. One week after imposing MTBE, a marked increase in enzyme activity was recorded in the plants treated with 20 and 40ml/L.MTBE .The percent increase was 4% and 32% respectively, also a slight increase in activity was recorded in plants subjected to 60ml/L. MTBE comparing with the control plants, the increase accounted only to about 2 % .Plants subjected to 80ml/L. MTBE, however, showed a significant reduction of PEPCase (63%).

Discussion:

Very little is known about the direct effects on plants of volatile organic compounds (VOCs) especially MTBE. Because MTBE is highly water soluble, it is readily absorbed by plants. For this reason, plant growth

was expected to be a sensitive endpoint. In this investigation, shoot length was more reduced in MTBE treated plants than root length. Leaf area as a growth parameter was also affected by MTBE. These observations suggest that MTBE might be transported within the plant from roots to shoots. In addition it might give some indication that the site of toxic action is the leaf (confirming our leaf area data) where transpiration, photosynthesis and active cell growth occur. Youn-Joo and Woo-Mi (2007) reported that the growth of all the plants tested (*Phaseolus radiatus*, *Cucumis sativus*, *Triticum aestivum*, *Zea mays*, *Sorghum bicolor*, and *Brassica alboglabra*) was adversely affected by Tert-Butyl alcohol (TBA) and MTBE. They also reported that MTBE is more toxic than TBA to most of the test species. Consistent findings of earlier work under laboratory conditions reported negative growth responses of selected algae to MTBE (Rousch and Sommerfield, 1998). Recent studies on (VOCs) including MTBE reported significant effects of MTBE (and related VOCs) on leaf water content and photosynthetic efficiency of some plant species (Cape *et al.*, 2003). Our data agree with the data obtained by Youn-Joo *et al.* (2002) who reported a reduction in seed germination, shoot and root growth of 4 plant types (oats, sweet corn, wheat, and lettuce) subjected to different MTBE concentrations in the soil. Xiao-Zhang and Ji-Dong (2006) investigated the uptake, metabolism and toxicity of MTBE in *Salix babylonica*, they reported that almost all applied MTBE was removed from the hydroponic solution by the plants. Small amounts of MTBE were detected in the plant tissues, but a large fraction was found in the air through plant transpiration. PEP carboxylase is an important key enzyme in all plants and has been the subject of extensive research in the last few decades. PEPCase activity in plants were stimulated by many environmental stresses including salt and water stress, ion deficiency, light, CO₂ concentration, heavy metals, air and soil pollutants (Tietz and Wild, 1991; Soussi *et al.*, 1998; Murchie *et al.*, 2000; Jeanneau *et al.*, 2002; Bailey *et al.*, 2006; Murmu and William, 2007) Those authors reported that a direct relationship between the stress imposed and stimulation of PEP carboxylase activity. Our results, however, support this relationship only in part: the enzyme activity increased markedly when concentrations of 20 and 40ml/L. MTBE were imposed in the nutrient medium, but decreased sharply at the highest concentration used (80ml/L). Therefore the induction of maize PEPCase in the presence of MTBE is a factor of concentration of this compound in the nutrient medium. The typical concentration of MTBE detected in ground water (Moran *et al.*, 2000) and surface water (An *et al.*, 2002) are generally low (e.g., < 1µg/L). The levels of MTBE detected in a water body may be too low to have an adverse influence on the plants when utilized for agricultural purposes over a short term. Because the present study was limited to a short-term toxicity test (7-days period), it only indicates a long-term adverse effect of MTBE to plants. Also lower levels of MTBE may show chronic adverse effects after long-term exposure.

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