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Phytotoxicity of Methyl tert-butyl Ether to Common Bean (*Phaseolus vulgaris* L.) Plants

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Abstract: The current investigation was conducted to report on the phytotoxicity of methyl tert-butyl ether (MTBE) to common bean (*Phaseolus vulgaris* L. cv. Nebraska) plants. The two-week-old potted plants were subjected to four weekly soil applications of aqueous MTBE concentrations (0, 1, 10, 25 and 50 ml L^{-1}). The root growth, flower and pod development were more sensitive to MTBE treatments; while, stem growth and photosynthetic pigments were more persistent to the toxicity of MTBE. The total number of protein bands/lane in SDS-PAGE protein profile was reduced by MTBE treatments. Two proteins of molecular weight 53.83 and 30.96 kDa were newly synthesized at the highest concentrations (25 and 50 ml L^{-1}) of MTBE; while the syntheses of other proteins were completely inhibited with varying sensitivity to MTBE concentrations. The toxicity of MTBE concentrations caused progressive collapsing of epidermal and cortical tissues of the plant roots. MTBE is quite toxic to crop plants in contaminated soils of agricultural systems.

Key words: MTBE, phytotoxicity, common bean, SD-PAGE protein

INTRODUCTION

MTBE (methyl tertiary-butyl ether) is a chemical compound that is manufactured by the chemical reaction of methanol and isobutylene. MTBE is produced in very large quantities (over 200,000 barrels per day in the US in 1999) making it the second on the list of gasoline contained oxygenates in the US (Johnson *et al*., 2001). It is almost exclusively used as a fuel additive in motor gasoline. It is one of a group of chemicals commonly known as oxygenates because they raise the oxygen content of gasoline. At room temperature, MTBE is a volatile, flammable and colorless liquid that dissolves rather easily in water.

Over the past several decades, MTBE as additive to gasoline intended to either boost ratings of fuel or to reduce air pollution has been accepted worldwide. Since 1992, MTBE has been used at higher concentrations in some gasoline to fulfill the oxygenate requirements set by Congress in the 1990 Clean Air Act Amendments.

The cause of MTBE leaking into the environment is mainly attributed to gasoline spills and leaks from pipelines, underground and aboveground storage tanks and transport accidents (An *et al*., 2002). Due to its high solubility in water and low sorption tendency in soils, MTBE can rapidly penetrate the soil layer and enter the groundwater shortly after the spill. It has become one of the most problematic pollutants in urban soils and groundwater worldwide (Franklin *et al*., 2000).

Meanwhile, MTBE is quite persistent to abiotic decomposition, e.g., 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). The toxicity of MTBE to animals and humans is well documented. The US EPA has classified MTBE as a possible human carcinogen (Squillace *et al*., 1996).

Toxicity of MTBE to algae, invertebrates and fish has been intensively studied by Bentsson and Tarkpea (1994) Benkinney *et al*. (1994), Werner *et al*. (2001) and Rausina *et al*. (2002). Very few works, however, have been conducted on MTBE toxicity to terrestrial plant and economic food crops. Lately, Beltagi *et al*. (2006) reported on negative growth responses of germinating wheat seedlings to MTBE. Thus, the objective of the current study was to assess the anabolic growth responses of common bean (*Phaseolus vulgaris* L.) plants to the toxic effects of MTBE in the soil, with a special emphasis on its toxicity to SDS-PAGE proteins.

MATERIALS AND METHODS

Plant material: The experimental plant used in the current study was pure strain of common bean (*Phaseolus vulgaris* L. cv. Nebraska). The seeds were obtained from the Agricultural Research Center in Giza, Egypt.

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Experimental design: The experiment was conducted in the Botanical Garden of Botany Department, Faculty of Science, Ismailia, Egypt during the spring of 2006. For plantation, 25 plastic pots (20 cm) were filled with homogenous pre-sieved garden soil (loamy sand). Seeds were soaked in the pot soil about 3 cm deep and all pots were watered up to saturation, then kept in the open garden and irrigated regularly to field capacity until MTBE treatments.

Treatments: After two weeks from soaking, the planted pots were randomly subdivided into five equal groups (5 pots each). One group was treated with pure water and sampled as control. The other four groups were subjected to four weekly doses (50 mL) of MTBE concentrations (1, 10, 25 and 50 ml L^{-1}) added to the soil with 50 mL of half-Hoagland solution.

Sampling and measurements

Growth vigor: After eight weeks from soaking, vegetative and reproductive growth parameters (shoot and root lengths, fresh and dry weights, leaf, flower and pod numbers) were recorded. All parameters were statistically analyzed by multiple comparison procedure at 5% significance level ($p = 0.05$) using t-test and mean separation by Least Significant Difference (LSD) (Steel and Torrie, 1980).

Chlorophyll pigments: Chlorophyll a, chlorophyll b and carotenoids were estimated in the fresh leaves (μg mL^{-1}) following the method of Lichtenthaler and Wellburn (1983).

Protein electrophoresis: Extraction of total protein Total protein extracts were prepared by extracting appropriate weight from the frozen plant material with 0.125 M Tris/Borate, pH 8.9. All the obtained extracts were kept at 4°C for 24 h and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for electrophoresis.

Gel electrophoresis: SDS polyacrylamide gel electrophoresis (PAGE) was carried out with gel slabs according to the method of Laemmili (1970). Protein subunit bands were stained with coomassie blue R-250 by standard techniques. The gel was scanned using Gel-Pro-Analyzer ver. 3.3 (Media Cypermetics, 93-97).

Histological examination: From the 6-week-old plants, root specimens were fixed in formalin: acetic acid: ethanol (FAA). Tissues were dehydrated in n-butyl alcohol, infiltrated and embedded in pure paraffin wax (m.p. 56-58° C) as described by Johansen (1940). A rotary microtome was used to prepare serial sections (10 μ), which were then stained with safranin and light green or hematoxilin. Stained sections were examined and photographed with Zeiss Microscope.

RESULTS

The growth and reproductive parameters (Table 1) of common bean plants revealed significant stimulation of vegetative growth but inhibition of the number of flowers by the lowest concentration (1 ml L^{-1}) of MTBE. However, the highest concentration (50 ml L^{-1}) of MTBE resulted in significant reduction in root growth (length, fresh weight and dry weight) but not in the stem growth. The numbers of leaves, flowers and pods per plant responded dramatically to almost all concentrations of MTBE. On the other hand, the content of photosynthetic pigments in the foliage leaves increased in response to MTBE treatments (Fig. 1).

The SDS-PAGE protein profile (Fig. 2) of common bean plants indicates obvious variations in banding patterns in response to MTBE treatments. MTBE induced qualitative (appearance and/or disappearance of protein bands) and quantitative (band%) changes in the polypeptide molecular weights (Table 2). The total number of protein bands/lane was reduced by all MTBE treatments. Two molecular weight polypeptides (53.83 and 30.96 kDa) were newly synthesized at the highest concentrations (25 and 50 ml L^{-1}) of MTBE. The syntheses of other molecular weight polypeptide were completely inhibited with varying sensitivity to MTBE concentrations.

Table 1: Mean vegetative and reproductive growth parameters of common bean (*Phaseolus vulgaris* L. cv. Nebraska) plants treated with different concentrations of MTBE

	Vegetative and reproductive growth parameters								
Treatments	Stem length (cm)	Stem fresh weight (g)	Stem dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	No. of leaves $plant^{-1}$	No. of flowers $plant^{-1}$	No. of pods $plant^{-1}$
C	51.000	15.670	2.470	16.530	8.570	0.627	16.330	22.000	13.670
T_1	71.670	24.430	3.730	14.100	17.730	0.923	12.670	17.000	12.000
T_2	54.330	17.730	3.360	11.470	7.270	0.620	12.670	16.330	9.330
T_3	53.000	16.590	3.340	11.330	6.600	0.550	12.330	14.330	8.330
T_4	48.330	13.370	2.940	9.100	3.370	0.440	10.330	8.670	2.670
LSD	9.228	4.162	0.480	2.863	5.395	0.179	2.152	4.824	4.220

($p = 0.05$), C = Untreated (controlled) plants T_1, T_2, T_3 and T_4 = Plants treated with 1, 10, 25 and 50 ml L^{-1} of MTBE

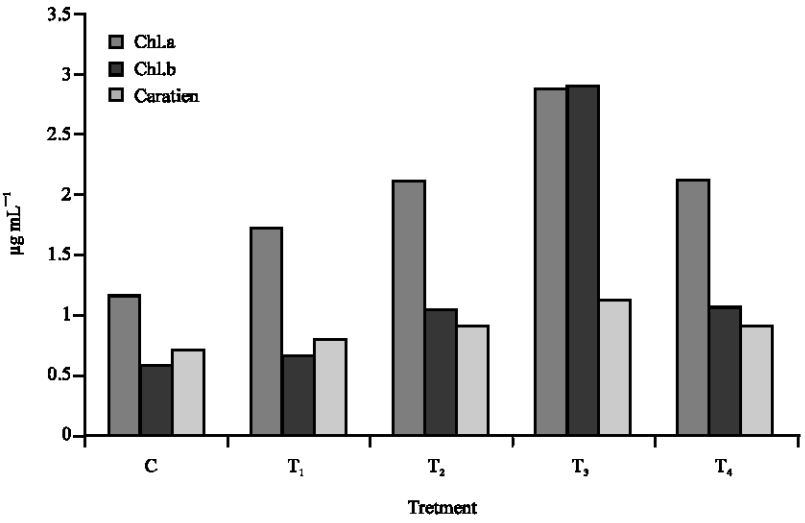

Fig. 1: Photosynthetic pigments (µg mL^{-1}) in folige leaves of common bean (*Phaseolus vulgaris* L.) plants. C, untreated (controlled) plants; T_1, T_2, T_3 and T_4, plants treated with 1, 10, 25 and 50 ml L^{-1} of MTBE

Table 2: Comparative analysis of relative concentration, molecular weight (M. Wt.) and mobility rate (R_m) of protein profile in common bean (*Phaseolus vulgaris* L.) plants treated with different MTBE (methyl tert-butyl ether) concentrations

	Treatment and Band (%)						
Band No.	1	2	3	4	5	R_m	Mol. wt. (kDa)
1	0.26	0.95	0.30	0.18	0.84	0.07	352.74
2	1.10	0.84	0.22	0.54	1.56	0.09	291.51
3	0.52	4.43	0.10	-	-	0.12	233.37
4	1.44	1.44	4.45	4.80	4.72	0.15	180.99
5	0.36	-	-	1.05	1.13	0.17	175.33
6	0.03	-	-	-	-	0.19	144.90
7	0.45	-	-	-	0.67	0.22	114.17
8	1.17	0.89	2.00	2.89	-	0.24	103.79
9	0.29	-	-	-	-	0.28	89.86
10	1.01	1.87	1.84	2.52	1.14	0.31	83.27
11	1.82	-	0.82	0.71	0.83	o.34	75.72
12	2.59	2.16	2.76	2.43	2.18	0.38	69.62
13	0.77	1.17	1.25	0.72	0.90	0.41	65.40
14	2.52	4.42	3.07	1.73	1.73	0.45	57.21
15	-	-	-	3.54	4.31	0.47	53.83
16	13.70	11.30	11.00	4.90	6.48	0.49	51.28
17	3.32	2.90	-	-	-	0.53	45.13
18	4.46	4.05	5.08	2.73	3.85	0.59	39.00
19	7.72	4.33	4.58	3.18	4.13	0.62	36.64
20	4.58	3.78	3.20	3.30	3.67	0.65	35.33
21	5.51	5.56	5.30	5.77	4.96	0.71	33.03
22	2.36	1.74	2.71	2.67	1.47	0.74	31.78
23	-	-	-	2.84	2.29	0.76	30.96
24	2.63	3.73	3.59	3.66	3.53	0.79	30.17
25	3.04	2.54	2.47	5.27	3.37	0.82	29.02
26	1.08	0.57	0.66	0.39	0.34	0.86	27.70
27	1.91	1.41	1.17	0.71	0.37	0.88	26.92
28	0.46	0.44	0.31	0.33	0.21	0.90	26.23
29	0.32	0.34	0.47	1.69	0.62	0.93	25.43
30	16.40	24.30	23.50	22.70	19.40	0.96	24.46
Bands/lane	28.00	23.00	22.00	25.00	25.00		

1 = Untreated (control) plants 2, 3, 4 and 5 = Plants treated with 1, 10, 25 and 50 ml L^{-1} of MTBE, - = No band

The thin sections prepared from common bean roots (Fig. 3) of MTBE-treated plants indicated progressive collapsing and disruption of the epidermal and cortical tissue layers of the roots as the concentration of MTBE increased (Fig. 3b-d) compared to the root of untreated plants (Fig. 3a).

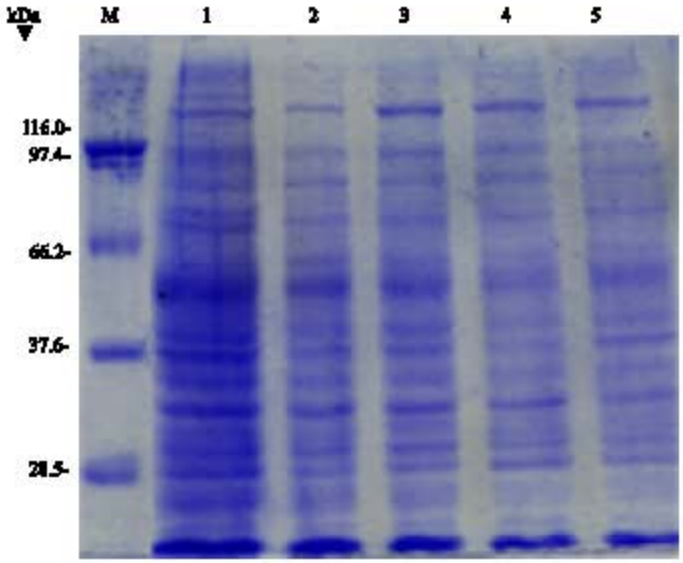

Fig. 2: Electrophotograph of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of the total proteins of common bean (*Phaseolus vulgaris L.*) plants. 1, untreated (controlled) plants (Track 1); 2, 3, 4 and 5, plants treated with 1, 10, 25 and 50 ml L^{-1} of MTBE (Tracks 2, 3, 4 and 5). M, molecular weight markers used on Polyacrylamide gel

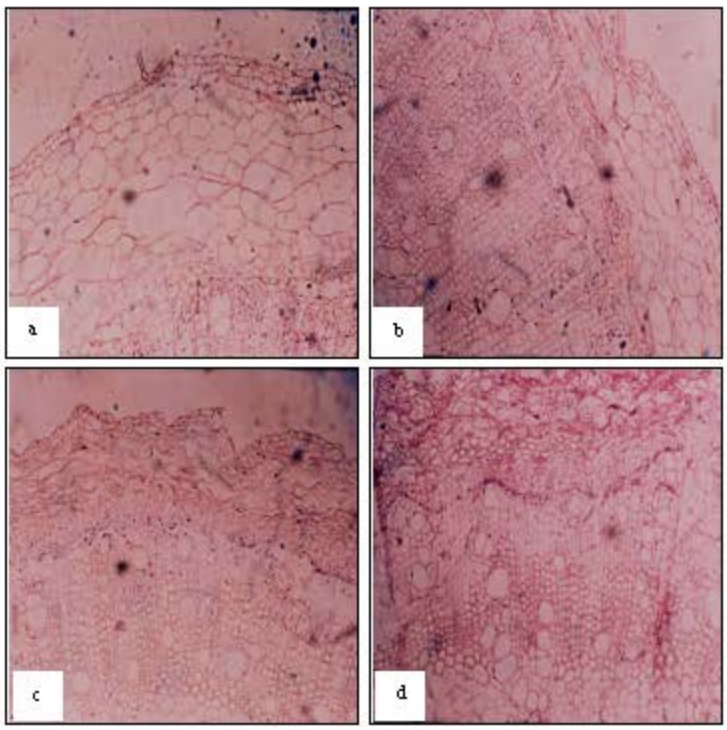

Fig. 3: Deteriorative effect of MTBE on the integrity of the epidermal and cortical tissues of common bean (*Phaseolus vulgaris* L.) plant root in untreated (a) plants and treated (b, c and d) with 10, 25 and 50 ml L^{-1} of MTBE

DISCUSSION

The responses of vascular plants to organic chemicals were grouped into three categories: unique features, common features and growth parameters (Nellssen and Fletghe, 1993). In this investigation, a generalized inhibitory effect of MTBE to common bean plants was noticed. Consistent findings of earlier work under laboratory conditions reported negative growth responses of selected algae to MTBE (Rousch and Sommerfeld, 1998). Recent studies on Volatile Organic Compounds (VOC) reported significant effects of VOC (including MTBE) on leaf water content and photosynthetic efficiency of some plant species (Cape *et al*., 2003). In weeping willow (*Salix babylonica* L.), severe toxic symptoms were detected after a short time period (120 h) as shown as significant reduction (35%) in transpiration, but chlorosis of leaves was not observed in all treatment groups for the whole duration of the test (Yu and Gu, 2006).

Holding fairly to our observations, the persistency of stem growth and photosynthetic pigments in common bean plants under MTBE stress could be due to the low MTBE concentration in the leaves of the plants, plant ability in removing MTBE in the soil through transpiration and restoring of physiological functions of the plants (Yu and Gu, 2006). On the other hand, the higher sensitivity of common bean root growth to MTBE might be attributed to the greater accumulation of MTBE by plant roots rather than stems.

The stressful environment of MTBE treatment which resulted in quantitative changes in the banding patterns of protein profile of common bean plants might be resulted from the subfractionation of protein bands caused by the phytotoxicity of MTBE (Shehata *et al*., 2000; Beltagi *et al*., 2006). As a result, new bands with altered mobility were formed instead of the final end product original protein (Abdel-Salam *et al*., 1996). On the other hand, the syntheses of new polypeptides as induced by MTBE treatment might be a participation in the strategy of detoxification of MTBE by the plants (Beltagi *et al*., 2006).

The most interesting effect of MTBE as characterized as an aliphatic hydrocarbon ether with high solubility in water (Johnson *et al*., 2000) and quite persistent to abiotic decomposition (Fayolle *et al*., 2001), MTBE treatment caused collapsing of epidermal and subepidermal (cortical) tissues of the MTBE-treated common bean roots. This was because of its dissolving effect on pectic materials of the middle lamellae of these tissues and its anesthetic effect on the permeability of the cell membranes as well. Accordingly, future work dealing with MTBE phytotoxicity should be directed to plant root responses to MTBE.

In conclusion, due to its high water solubility, low partition tendency and low biodegradability, MTBE after the spill can rapidly penetrate the soil layer and enter the plants resulting in negative impacts on agricultural crops.

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