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GERMINATION AND GROWTH PARAMETERS OF *RAPHANUS SATIVUS* L. AND *TRITICUM SATIVUM* L. PLANTS EXPOSED TO TNT AND HMX EXPLOSIVES

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SUMMARY

Differences in the degree of expressed phytotoxicity due to various 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX)TNT and HMX-concentrations were established. Effects of TNT and HMX on germination, seedling development and some basic growth components include relative growth rate (RGR), leaf area ratio (LAR) and specific leaf area (SLA), ear and root tuber fresh weight of *Raphanus sativus* L. and *Triticum sativum* L plants were characterized. Growth analysis of TNT and HMX-treated plants showed inhibiting effect of TNT on *Raphanus sativus* L., *Triticum sativum* L. and HMX on *Raphanus sativus* L., while HMX cause increasing growth parameters to *Triticum sativum* L. up to 37.5% relative to control samples. Results indicate *Raphanus sativus* L. is less tolerant to TNT and HMX than *Triticum sativum* L.

Key words: **Germination, growth parameters, HMX, *Raphanus sativus* L., TNT, *Triticum sativum* L.**

INTRODUCTION

2,4,6-Trinitrotoluene (TNT) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX), is used as a high explosive in military armaments and have been released into the environment from munitions production and processing facilities resulting in their dissemination into the environment. Their presence in waterways and soil poses ecological and health hazards. High concentrations of 2,4,6-trinitrotoluene (TNT) and other contaminants are commonly found in former areas of explosives loading, handling and packaging. HMX is commonly present in explosive-contaminated soil and groundwater (Griest *et al*. 1993, Simini *et al*. 1995, Hovatter *et al*. 1997, Talmage *et al.* 1999). The environmental concentrations of HMX in soil on contaminated sites can range from 0.7 to 5700 mg kg^{-1}, whereas the groundwater and surface water ranged from 1.3 to 4200 and 1.9 to 67 mg l^{-1}, respectively (Talmage *et al.* 1999). This explosive is toxic to some aquatic organisms, including fathead minnows (*Pimephales promelas*) and the fresh-water micro-crustacean (*Daphnia magna*) (Talmage *et al.* 1999).

Raphanus sativus L. and *Triticum sativum* L. are the predominant species cultivated on the farm site. The number of studies describing the phytotoxicity of explosives, such as TNT, HMX, and HMX, on higher plants is limited (Elly *et al.* 2006, Frische and Höper 2003, Sunahara *et al.* 2001, Krishnan *et al.* 2000, Talmage *et al.* 1999, Thorne 1999, Sens *et al.* 1999, Hughes *et al.* 1997). TNT-contaminated effluent reduced growth of mixtures of tall fescue, ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.), orchardgrass (*Dactylis glomerata* L.) and ryegrass (Palazzo and Leggett 1983). Schott and Worthley (1974) reported reduction in duckweed (*Lemna perpusilla* Torr.) growth after exposure to 1 mg TNT $litre^{-1}$ and the plants were killed

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at concentrations exceeding 5 mg TNT liter^{-1}. Palazzo and Leggett (1986) reported reduction in the dry weights of yellow nutsedge (*Cyperus esculentus* L.) leaves, roots, and rhizomes following exposure to 5 to 20 mg TNT liter^{-1}.

Growth on the whole plant level is an integral physiological process with a higher degree of organization and regulation compared to the other cardinal physiological process. Thus it is a good approach to follow a logical sequence of steps in investigating plant growth limitations. Some basic growth components (Vassilev and Yordanov 1997) include RGR (relative growth rate), LAR (leaf area ratio), and SLA (specific leaf area), which were investigated in this research.

Our objective was to determine the effect of field soils previously contaminated with TNT and HMX on germination and growth parameters of *Raphanus sativus* L. and *Triticum sativum* L. In this work, plant seeds were exposed to TNT and HMX for 10 days, and seed germination was determined. *Triticum sativum* L. and *Raphanus sativus* L. were exposed for 75 and 45 days respectively, and survival and growth parameters were recorded. Long-term exposure tests were conducted to evaluate sub lethal toxicity with TNT and HMX as the main contaminant.

MATERIALS AND METHODS

Non-contaminated soil was obtained from loamy soil of Varamin (a town, near to Tehran, Iran). Characteristics of uncontaminated soil were performed in triplicate as per soil survey staff (1984). The soil pH, organic matter content and cation exchange capacity were respectively 6.9, 0.53% and 580 ppm. Experiments were conducted with plants growing in contaminated and noncontaminated soil mixed at different ratios to obtain a range of TNT and HMX concentrations. Noncontaminated soil was spiked in concentrations ranging from 0 to 500 mg TNT and 0 to 650 mg HMX per Kg soil. Noncontaminated soils with plants were included as controls. TNT and HMX were prepared from local industrials and other chemicals used were of analytical grade and obtained from E. Merck (Darmstadt, FRG).

Germination and seedling growth: Artificial soils were spiked with 0, 50, 200, 350 and 500 mg TNT and 0, 50, 200, 350, 500 and 650 mg HMX kg^{-1} DW using acetone as a solvent. Solvent-spiked artificial soils served as controls. After spiking, the soils were mixed with a stainless steel spatula and placed in a vented fume hood without illumination for 2 h to allow the acetone evaporation prior to exposure of the test organisms. All units were sprayed with reverse osmosis (RO) water immediately after the test organisms were placed on the soils, and, subsequently, every other day as needed. *Triticum sativum* L. and *Raphanus sativus* L. seeds (from PVR. Seed and Plant Certification Research Institute, Tehran, Iran) were surface-disinfected by immersing in 0.3% sodium hypochlorite for 5 min and rinsing with sterile, deionized distilled water. For the plant test units, 25 seeds were placed on 5 g of soil contained in a 15-mL petri dish. Units were incubated in a walk-in growth chamber illuminated with 500–600 µE m^{-2}s^{-1} at the seed surface at a 14-h photoperiod, temperature of 22–26 °C and *ca* 99% relative humidity. The parameter for plant response was seed germination, observed as root emergence at the end of 10 days, which was long enough to observe acute toxicity. In each treatment, the number of newly emerged radicles, indicating germination, was recorded daily for 10 days and converted to germination capacity (percentage of seed capable of completing germination in 10 days (Faulkner and Harvey 1981, Bewley and Black 1994). Treatments were replicated six times and the experiment repeated two times. In these analyses, a p-value <0.05 was accepted as significant.

Growth components: For the plant test units, 10 seeds were planted in plastic pots containing 1 kg of spiked soil. The pots were watered daily and kept in botanical garden under natural photoperiod of 12–13 h and temperature of 28 ± 4^0C. Care was taken to avoid drainage of soil solution during the treatment by giving water slightly less than the field capacity and draining water, if any, was returned to pots. *Triticum sativum* L. and *Raphanus sativus* L. were exposed for 75 and 45 days after sowing respectively, and survival and growth parameters were recorded. Plant growth response to TNT and HMX treatment include RGR, LAR, and SLA were determined using Evans and

Hughes methods (1962). Treatments were replicated six times and the experiment repeated two times. A completely randomized design was used in these experiments with *Triticum sativum* L. and *Raphanus sativus* L. grown side by side, but in separate pots. Values are means from six replications. In these analyses, a p-value <0.05 was accepted as significant.

Root tuber or ear fresh weight: *Triticum sativum* L. and *Raphanus sativus* L. were harvested 45 and 75 days after planting. *Triticum sativum* L. ear and *Raphanus sativus* L. root tuber were separated and adhering soil to root was removed by washing, then, root and ear fresh weight were recorded.

RESULTS

Germination: *Triticum sativum* L. and *Raphanus sativus* L. radicles began emerging 10 days after sowing (DAS) in each experiment; shoot emergence began within 12 DAI. The results are summarized in Table 1 and Figs. 1-2 indicate that germination percent and seedling survival declined in presence of TNT and HMX. Seedling survival percent was similar to germination percent in both plant species (Fig. 2).

Table 1. Percent change in plant growth response* of *Raphanus sativus* L. and *Triticum sativum* L. to TNT and HMX treatment between control samples and 500 mg of HMX and TNT Kg^{-1} soil.

Growth parameters	*Raphanus sativus* L.		*Triticum sativum* L.	
	HMX	TNT	HMX	TNT
Germination (%)	-31.63	-67.01	-5.41	-61.86
Seedling survival (%)	-36.73	-72.45	-5.76	-72.16
LAR [$m^2.kg^{-1}$ (plant)]	-26.48	-57.28	19.22	-32.20
SLA [$m^2.kg^{-1}$ (leaf)]	-27.73	-34.40	19.32	-31.84
RGR [$g.kg^{-1}.day^{-1}$]	-89.15	-99.47	49.34	-98.93
Root tuber or Ear fresh weight	-69.96	-96.60	37.32	-86.75

*Values are means of 6 replications; p<0.05.

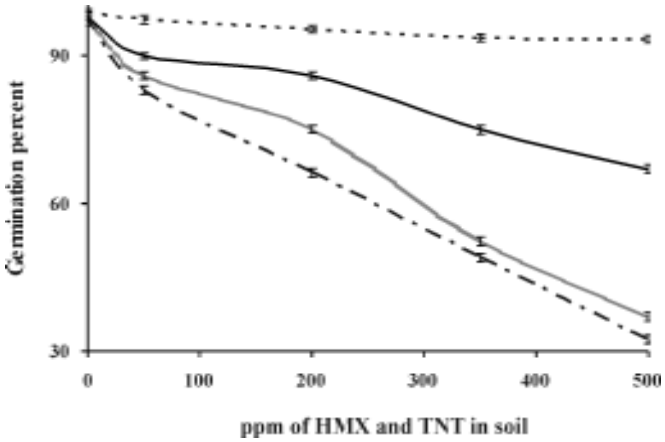

Fig. 1. Mean germination capacity of *Triticum sativum* L. and *Raphanus sativus* L. seeds after 10 days exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT
Vertical bars represent Fisher's least squares difference (LSD) values (0.05).

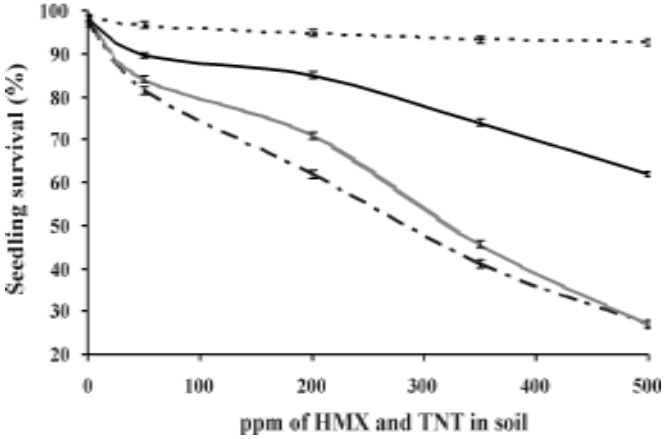

Fig. 2. Mean seedling survival (%) of *Triticum sativum* L. and *Raphanus sativus* L. after 12 days exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT

Plant growth response to TNT and HMX treatment: The results are summarized in Table 1 and Figs. 3-5. Table 1 shows inhibition percent of growth parameters in *Raphanus sativus* L. subjected to a 45-day treatment and *Triticum sativum* to 75 day treatment with TNT and HMX (500-ppm) compared to control samples. Results from experiments conducted in spiked soils indicate that *Raphanus sativus* L. and *Triticum sativum* L.

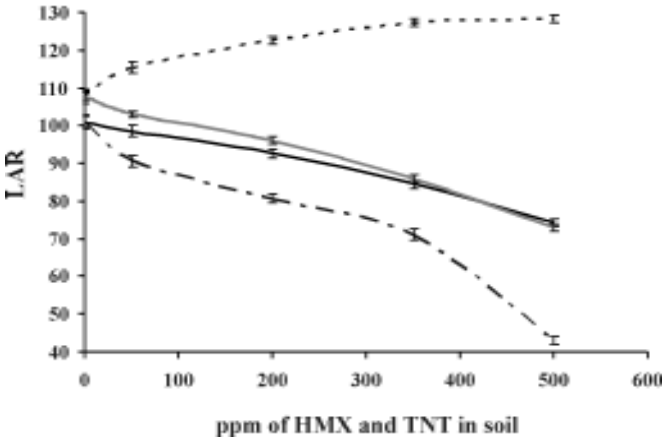

Fig. 3. Mean LAR of *Triticum sativum* L. and *Raphanus sativus* L. at 75 and 45 days after sowing respectively, exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT

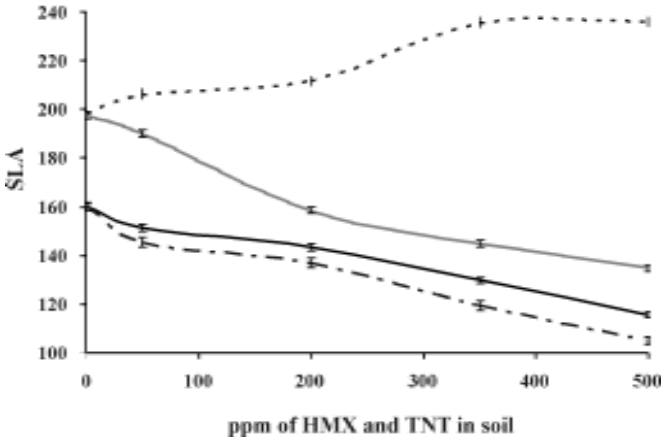

Fig. 4. Mean SLA of *Triticum sativum* L. and *Raphanus sativus* L. at 75 and 45 days after sowing respectively, exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT

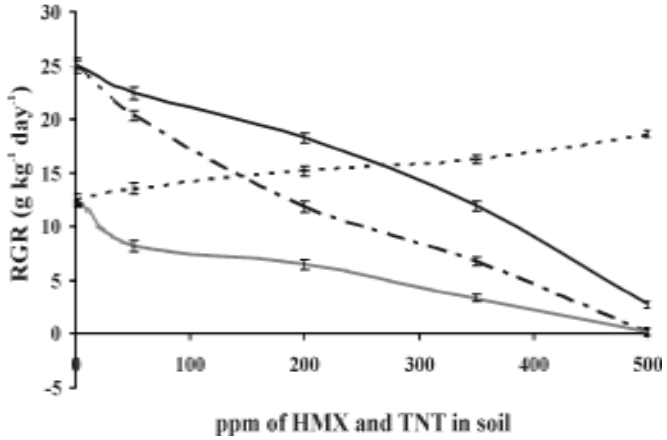

Fig. 5. Mean RGR of *Triticum sativum* L. and *Raphanus sativus* L. at 75 and 45 days after sowing respectively, exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT

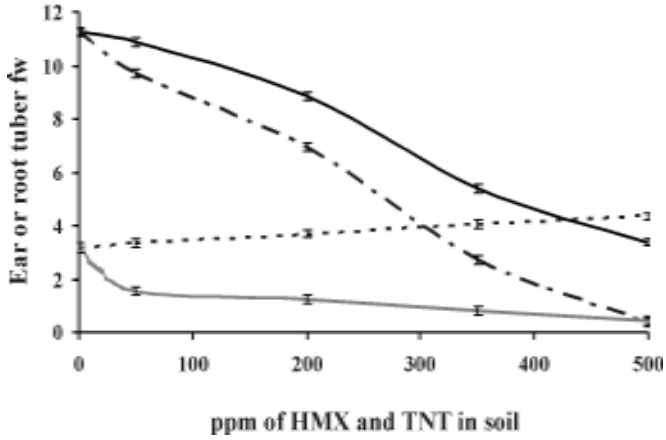

Fig. 6. Mean root tuber or ear fresh weight of *Triticum sativum* L. and *Raphanus sativus* L. at 75 and 45 days after sowing respectively, exposure to five TNT and HMX concentrations. —— *Raphanus sativus* L.-HMX, —— *Triticum sativum* L.-HMX, ▬▬ *Raphanus sativus* L.-TNT, —— *Triticum sativum* L.-TNT

establishment may be limited on soil highly contaminated with munitions compounds.

Root tuber or ear fresh weight: Recoverable root tuber fresh weight was reduced by 70% in *Raphanus sativus* L. and the ear fresh weight was reduced by 86% in *Triticum sativum* L. at 500 mg TNT kg^{-1} soil. At 500 mg HMX kg^{-1} soil, the tuber fresh weight was reduced by 96% in *Raphanus sativus* L., while ear fresh weight in *Triticum sativum* L. increased by 37.5% relative to control samples.

DISCUSSION

Differences in the degree of expressed phytotoxicity due to various TNT and HMX-concentrations were established. Germination of both plant species decreased with increasing TNT and HMX concentration [Fig. 1], but germination could not occur at 650 ppm of HMX in both plant test species. *Raphanus sativus* L. and *Triticum sativum* L. germination was reduced by more

than 60% at TNT concentration of 500 mg l^{-1}, while *Raphanus sativus* L. germination was reduced only by 31% until soil solution concentrations were 500 mg HMX l^{-1} or greater. No significant differences were observed between 0 and 50 ppm HMX treatment to *Raphanus sativus* L. after 12 days. *Triticum sativum* L. germination did not decline significantly as HMX concentration increased in the soil. Because of higher solubility of TNT in water (150 mg TNT L^{-1}) than HMX (5 mg HMX L^{-1}) (Michael 2007, Rosenblatt *et al.* 1991), toxic effects of TNT was higher than HMX and therefore, reduction of germination percent was sharper with TNT contamination. In addition, linear response of germination to TNT and HMX reflects the importance of concentration on toxic response.

TNT and HMX-treated plants (Table 1) showed inhibition of growth. In *Raphanus sativus* L. the growth characters including LAR, SLA, RGR and root tuber fresh weight decreased due to TNT and HMX treatment. HMX caused increase in growth of *Triticum sativum* L. but TNT reduced the growth parameters of this plant including LAR, SLA, RGR and ear fresh weight. The reduction of LAR could be a consequence from SLA. Inhibitions of LAR and SLA were related to the negative effect of TNT and HMX on SLA with disorders in water supply. Although the mode of action for TNT phytotoxicity is unknown, it affects the growth rate. TNT appears to affect tissue differentiation and respiration in the roots (Peterson *et al.* 1996).

The authors hypothesize that reduced cell turgor potential and cell-wall elasticity led to formation of smaller leaf cells and intercellular space area in TNT and HMX-treated plants except *Triticum sativum* L. subjected to a 75-day treatment with different HMX concentration up to 500-ppm in soil. This is may be due to contaminant-induced division, elongation and differentiation of cambium cells, a consequence from disturbed hormonal balance, but until now, there are no data supporting the suggestion. Both plant test species, *Raphanus sativus* L. and *Triticum sativum* L., tolerated TNT and HMX concentrations up to 50 mg dry weight (DW) during longer-term exposure. In the higher concentrations of HMX and TNT the plants were less tolerant and growth parameters reduce significantly. Over the experimental range of soil HMX concentrations, *Triticum sativum* L. exhibited greater tolerance to the presence of HMX than *Raphanus sativus* L. Growth parameters indicated that *Triticum sativum* L. tolerated TNT-contaminated soil better than of *Raphanus sativus* L. It has been shown that *Triticum sativum* L. when grown in HMX contaminated soil, not only tolerates HMX contamination but showed greater growth parameters than control samples. Results indicate *Triticum sativum* L. is more tolerant of TNT and HMX than *Raphanus sativus* L.

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