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Detection of Biotoxic Compounds with the 2,3,5-Triphenyltetrazolium Chloride Seed Germination Test

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The use of 2,3,5-triphenyltetrazolium chloride (TTC) as an indicator for the viability of seeds has been described by Lakon (1942 a,b). TTC is reduced by active dehydrogenases to a red formazan; thus the living parts of a plant embryo are stained. Necrotic regions remain unstained. The optimum conditions of this method have been evaluated by various authors (e.g., Cottrell 1948; Steiner and Werth 1984) and now the "topographic tetrazolium procedure" has been officially accepted by the International Seed Testing Association as a biochemical test for the viability of seeds (Anonymous 1966).

In the present study the applicability of the TTC test to detect various biotoxic compounds was tested with pea seeds and cereals.

MATERIALS AND METHODS

The seeds of the following agricultural plants were tested in comparison: brewing barley (Aura), wheat (Disponent), oat (Flemings Nova), maize (Gabit), rye (Danko and Halo), peas (Poneka, Stehgold and Haubners Siegerin). The indicator TTC (Serva, Heidelberg, Germany) was dissolved in phosphate buffer (pH 7) to produce a 1% solution. For the study of the influence of the biotoxic heavy metal ions Hg, Cu and Pb aqueous solutions with 0.005, 0.01, 0.05, 0.1, 0,5, 1 and 2 mg/100 mL of metal ions were prepared by using $HgCl_2$, $CuSO_4$ and Pb(II) acetate (Merck,, Darmstadt, Germany). As herbicides 0.1% aqueous solution (w/v) of Casoron (R) (dichlobenil) and 1% aqueous solution (w/v) of Basinex (R) (dalapon) (BASF, Ludwigshafen, Germany) were tested. The formulated materials were used. The tensides Marlipal (R) MG (alkylpolyglycolether, Marlophen (R) 89 (alkylphenolpolyethylene glycol ether) and Marlon (R) A 350 (sodium alkylbenzenesulfonate) were obtained from Hüls (Marl, Germany). 0.1 and 1% solutions (w/v) of these compounds were made with distilled water.

Treatment of seed embryos with biotoxic compounds. 30 grains of the individual seeds were soaked in one of the toxin solutions for 16 h. Controls were made in distilled water. All tests were made in triplicate.

After 16 h in the toxin solution, the peas were rinsed in distilled water. The seed coat was removed by cutting the coat opposite to the first rootlet, and the embryo was pressed out (Lakon 1950). The embryos were incubated in the TTC solution for 16 h at room temperature in the dark (the TTC solution is light sensitive). The embryos of barley, wheat and rye were removed with fine preparation needles, transferred into the TTC solution, and incubated for 7-8 h. Because of their thin seed coats, it was not necessary to remove the embryos of oat. 30 oat grains were cut in half with a sharp knife, and the half grains were incubated in the TTC solution for 36 h. The grains of maize were longitudinally cut in such a manner that the embryo was bisected. The incubation period in the TTC solution was 4 h.

Embryos of peas that fall into pieces after removal of the seed coat are not germinable (Lakon 1950). Not less than one half of the radicula and the connecting tissue between cotyledons and radicula must be stained when the peas are estimated to be germinable. When the plumula remains colorless, it should be freed from surrounding tissues and placed into the TTC solution once again. This additional treatment is necessary because it might be that the cotyledons were pressed so tightly in the first TTC treatment that too litle staining solution had come into contact with the plumula. When the plumula remains unstained even after the second TTC treatment the embryo is not germinable (Lakon 1950).

The estimation of germinability of cereal grains was performed according to the figures given by Eggebrecht (1949). Embryos are germinable when the shoot and a small part of the tissue bearing the root initials are stained. In the case of maize additionally at least one half of the scutellum must be stained when the embryos are considered to be germinable. In general the intensity of the staing is not important for the estimation of germinability.

RESULTS AND DISCUSSION

Copper(II) sulfate (Fig. 1). The germinability of maize, oat, barley and wheat was not inhibited by low concentrations of $CuSO_4$ (0.005, 0.01, 0.05, 0.1 and 0.5 g per 100 mL); higher levels had only minor effects. The germinability of peas (Poneka) and particularly of rye (Danko) was dictinctly reduced even by low levels

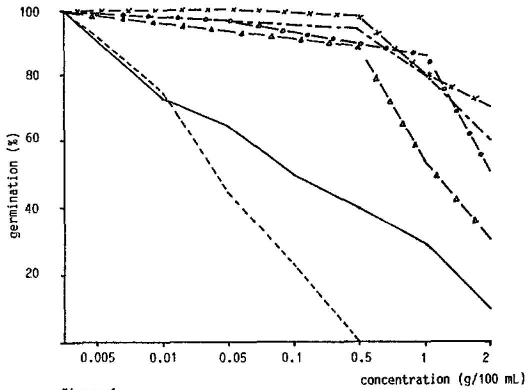

Figure 1

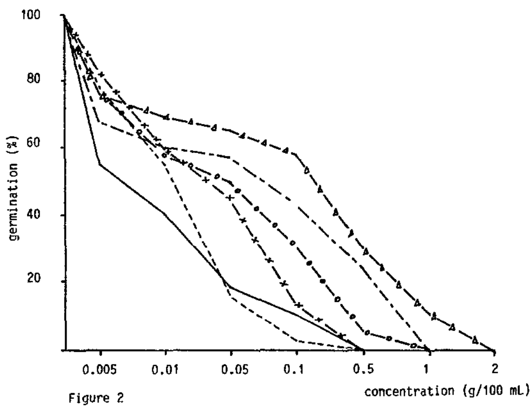

Figure 2

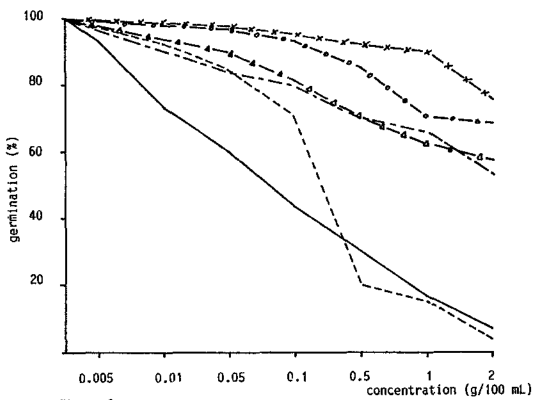

Figure 3

Figures 1-3. Influence of heavy metal salts on the germination of seeds. Fig.1: $CuSO_4$, Fig.2: $HgCl_2$, Fig.3: lead acetate.

——: pea (Poneka),
------: rye (Danko),
–×–: barley,
–o–: wheat,
–·–·–: oat,
–Δ–: maize.

(The values are in % to toxin-free controls)

(0.005 mg/100 mL).

Mercuric(II) chloride (Fig. 2). All test seeds were reduced in viability in an especially strong manner.

Lead (II) acetate (Fig. 3). The germinability of oat, maize, barley and wheat was reduced even by high concentrations only in a low degree. Rye (Danko) and peas (Poneka) reacted more sensitively.

Herbicides (Table 1). Peas (Poneka) were extensively reduced in viability by Casoron[(R)] (0.1 g/100 mL) and Basinex[(R)] (1 g/100 mL). Additionally, wheat seeds were sensitive towards Casoron[(R)] and rye towards Basinex[(R)].

Tensides (Table 1). Rye (Danko) was the only seed showing a high sensitivity towards Marlon[(R)] (1 g per 100 mL), Marlipan[(R)] (1 g/100 mL) and Marlophen[(R)] (1 g/100 mL).

Table 1. Germination of seeds under the influence of selected herbicides and tensides.

Compounds tested	Germination [(a)] (%) of peas (Poneka)	rye (Danko)	barley	wheat	oat	maize
Herbicides						
Casoron (0.1 g/100 mL)	26	61	74	42	74	56
Basinex (1 g/100 mL)	25	27	79	61	71	86
Tensides						
Marlon (1 g/100 mL)	80	21	84	83	52	76
Marlipal (1 g/100 mL)	85	18	87	95	78	90
Marlophen (1 g/100 mL)	87	26	95	85	108	97

(a) The values are based upon the germinability in toxin-free controls (= 100%).

Comparison of different seed cultivars.

Rye. Seeds of the variety "Danko" were more sensitive towards the heavy metal salts tested than those of the variety "Halo".

Peas. The results with the three varieties varied with the salts tested. "Stehgold" was most sensitive towards $CuSO_4$ and "Poneka" was especially sensitive to $HgCl_2$ and $Pb(CH_3COO)_2$.

The seeds of rye and peas showed the greatest sensitivity towards the heavy metal salts $CuSO_4$, $HgCl_2$ and Pb acetate. This observation is in good agreement with the results from agricultural studies demonstrating that rye is the most sensitive cereal species against changing soil quality factors (Frank 1976).

In summary the seed viability test with TTC can be used for screening and comparing the potency of environmental toxins, especially of heavy metal salts and of some herbicides. Rye and peas are especially suited for this quick and reliable test but differences in the sensitivity among several varieties must be considered.

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