

**BIOLOGICAL ACTIVITY OF HUMIC SUBSTANCES
AND THEIR FE-ENRICHED DERIVATIVES TOWARDS CUCUMBER PLANTS
UNDER FE-DEFICIENCY CONDITION**

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Humic substances (HS) are generally considered to increase availability of micronutrients such as Fe, Zn, Mg, Mn, Ca and others, low levels of which cause leaf yellowing called chlorosis. So, some HS preparation enriched with micronutrients are available in the agricultural market. The observed beneficial effect of HS is generally attributed to the chelating activity of HS that provides plants with micronutrients in easily assimilated form. However, HS are known to be universal bio-activators providing plant adaptation to stress of various nature. Therefore HS could be expected to mitigate negative effects of chlorosis due to their own biological activity. This study was aimed to compare influence of HS, their Fe-enriched derivative and commercially available iron supplier on cucumber plant growth under Fe-deficiency conditions.

Following preparation were used: potassium humate derived from coal (Solntsevskoe deposit, Sakhalin Island, Russia) assigned as K-Sh, its Fe-enriched derivative assigned as Fe-HS, and commercially available agricultural iron additive ferrous chelate of ethylenediaminedi(o-hydroxyphenylacetic) acid (Fe-EDDHA). The content of iron was measured using o-phenantrolin method after oxidative digestion and was as 1%, 9%, and 6% in K-HS, Fe-HS and Fe-EDDHA respectively.

To estimate biological activity of HS under iron deficiency conditions we applied bioassay technique. Plants of cucumbers *Cucumis sativus* L. were used as a target object. Cucumber seeds were grown in distilled water for 120 hours at 25°C in the dark followed by transferring seedlings into 0.05 mM solution of CaSO₄ for 24 hours. Then seedlings were transferred to plastic pots containing 0.11 of Hoagland's nutrition solution supplied with 25 µmol/l of iron and 15 mg/l of HS. Values of pH of all the solutions were adjusted to 8. The cucumber plants were grown with 12-hours light day. Hoagland's nutrition solution without Fe was used as blank.

After 32 days of growing plant photosynthesis efficiency were estimated in terms of the maximum activity of PSII (Fv/Fm) using pulse amplitude modulation (PAM) fluorometer (PAM-2000, Walz, Germany). Then cucumber plants were harvested and subjected to measuring shoots and roots length and weight and chlorophyll content. The content of iron in plant tissue was measured using o-phenantrolin method after oxidative digestion.

The obtained data showed that in the lack of iron in the nutrition media poor cucumber growth was observed. Plants demonstrated sings of chlorosis such as yellow or yellow-white leaves, poor photosynthesis efficiency (Fv/Fm = 0.45), and low chlorophyll a/b ratio (1.8). When iron was added in the form Fe-Sh or Fe-EDDHA no chlorosis symptoms was observed, and values of Fv/Fm and chlorophyll a/b ratio increased to 0.77-0.78 and 2.4-2.6. As a consequence, the biometric parameters of the treated plants – length and weight – were substantially increased as well (Figure 1).

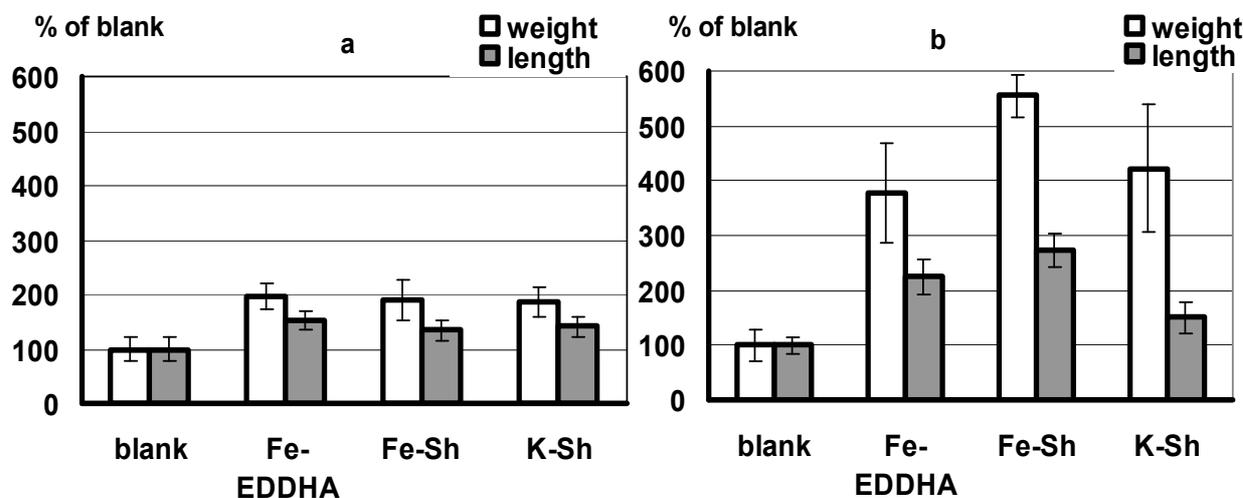


Figure 1. The influence of commercial iron chelate Fe-EDDHA, parent humic material K-Sh and its Fe-enriched derivative Fe-Sh on cucumber shoots' (a) and roots' (b) weight and length increase.

As it can be seen from the Figure 1, plants treated with Fe-EDDHA or Fe-Sh possessed higher weight and length of both shoots and roots as compared to blank. However, efficiency of studied iron suppliers did not differ one from another if shoots parameters were used as a target function, whereas substantial differences were observed for both length and weight of roots.

Of interest was that addition of K-Sh solely also led to a partial recovery of the Fe-deficient plants. In particular, the photosynthesis efficiency ($F_v/F_m = 0.77$) and chlorophyll a/b ratio (2.4) were significantly increased in humus-treated plants. At that, length and weight of cucumbers' shoots and roots were similar to those of plants treated with Fe suppliers.

Fe content in the leaves of treated plants were estimated as 508, 158, and 93 mg/kg of dry weight for Fe-EDDHA, Fe-Sh, and K-Sh respectively. The highest iron content was found in Fe-EDDHA treated plants whereas humics treated plants possessed much lower iron. The latter was evident for the fact that both Fe availability in the nutrition media and beneficial effect of HS were responsible for plants' recovery from Fe-deficiency chlorosis. So, mitigating activity of humic-based Fe additives under Fe-deficiency conditions resulted not only from the fact that plants could use Fe, but from protective activity of HS in relation to photosystem II either.