Effect of Sodium Cyanide on Wheat (*Triticum durum* cv. Altar and *T. aestivum* cv. Cumhuriyet)

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The effect of sodium cyanide on the morphology of stem, leaves and grain yields of *Triticum durum* cv. Altar and *Triticum aestivum* cv. Cumhuriyet grown under glass was studied. Seeds were planted in six different sets of pots containing ordinary garden soil. After formation of the first leaves, the first set was used as the control and watered using ordinary bottled water sold commercially. The other five sets with *T. durum* cv. Altar and *T. aestivum* cv. Cumhuriyet seedlings were additionally watered with various concentrations of sodium cyanide, the test-quantity used being 10-50 mg/L. Growth of individual plants was monitored until grain production. It was found that the sodium cyanide concentrations in the feed solutions affected plant stature, with the plants becoming progressively dwarfed with increasing dosage. Anomalies in the morphological and anatomical structure of the plant were also noted, as was early ripening of grain and sterility.

Key Words: Grain yields, Plant growth, Sodium cyanide, *Triticum aestivum*, *T. durum*, Wheat.

INTRODUCTION

Cyanide is the familiar name given to various compounds containing the cyanide anion (CN^-). Sodium cyanide (NaCN) is the salt of hydrocyanic acid (HCN). The toxic effects of cyanide compounds on living organisms is well-documented in literature. Cyanides are used or produced in sites where metallurgy, electrolysis, pesticide applications, tanning, metal-fabrication, iron works, photography, plating and fire extinguishing activities are carried out. The total amount of cyanide ingested by human beings from food and drink contaminated with cyanide is unknown. Smoking is probably the main source of cyanide inhalation for those not working in cyanide-related industries. Breathing smoke-filled air during fire is also a main source of cyanide exposure. Those living near garbage sites with dangerously high-level cyanide compounds are obviously more at risk than

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the general population. The Environmental Protection Agency (EPA) has formulated some rules concerning the maximum permitted amount of cyanide in drinking water which is 200 μg/L. The amount of HCN in dried beans, chick peas and hazelnuts varies from 25 to 250 ppm. Occupational Safety and Health Administration (OSHA) defined acceptable exposure to cyanide salts in a workplace should not be more than 5 mg/m^3. The maximum cyanide amount which workers can be exposed to HCN for 15 min is less than 4.7 ppm. HCN is a colourless gas with a low boiling point of 25.7 °C and a high vapour pressure of 100 kPa at 25 °C. As an asphyxiant, it prevents oxygen uptake by the tissues. The salts of HCN and many metal-cyanide complexes are far less toxic. While some cyanide compounds can be absorbed through the skin and mucous membranes, non-volatile cyanide salts, such as NaCN, are considered non-toxic if they are not ingested directly or come in contact with an open wound. It was discovered that the formation and vapourization of HCN due to hydrolysis, dissociation or photo-decomposition can be prevented by increasing the pH. In general, the term 'free cyanide' refers to both HCN and CN^-. The equilibrium relationship between CN^- and HCN is given by the following hydrolysis reaction:

$$CN^- + H_2O \rightleftharpoons HCN + OH^-$$

According to the above reaction, the relative proportion of the HCN and CN^- ions is dependent on the pH. The dissociation constant (K_a) of HCN is 6.17×10^{-10} at 25 °C. Therefore, at a pH value below 8, all the free cyanide will effectively be present as HCN. On the other hand, at a pH of 11.5, the ratio of HCN to CN^- will be about 0.005 or to express it in another way, 99.5 % of the cyanide will be in the form of CN^- rather than HCN. If the pH drops to 10.5, the ratio of HCN to CN^- will become 0.05. NaCN is a colourless compound with melting and boiling points of 564 °C and 1496°C, respectively. Its solubility is 82 g/100 g water. It possesses a high extraction affinity for gold and is thus widely used in advanced techniques for gold enrichment.

Cyanide has been used for long periods in Turkey and especially during the past 20 years in connection with gold-mining activities. The interaction of cyanide with the environment, cyanide toxicity, safety levels in air and water, have been investigated to some extent. The toxic effects of cyanide are well known and have been vehemently voiced by the country's protesting environmental groups and organizations. However, many local administrators insist that if proper safeguards are taken the concerns are unfounded. Thus gold mining was carried out *via* cyanide-application-techniques in Bergama, near Izmir in Western Turkey, for at least 2 years. One of the major problems in cyanide studies is that collected samples rapidly become unstable[1].

Due to the toxic effects of cyanide even at low concentrations cyanide is important as a pollutant-indicator. Cyanogenesis in plants, is a phenomenon which has been known for a long time and has attracted much interest from researchers. Based on 1994 data provided by FAO[2], the first 10 plants in a list of 23 species grown in the greatest quantities all over the world, are all cyanogenic. The purpose of our study is to observe and investigate the immediate effects of application of sodium cyanide on plants. Wheat (*Triticum durum* and *T. aestivum*) is an important staple crop and takes second place on the FAO list, after maize (*Zea mays*). Both the species of wheat selected for our studies are cyanogenic in leaf and more importantly, also in grain.

EXPERIMENTAL

The study was conducted in the plant physiology glasshouse of the Biology Department of Ege University, Izmir. For each species, 150 small-sized pots were used, a total of 300 pots for the experiment. In each pot, five seeds were planted. The pots were filled with ordinary calcareous garden soil (mean pH 8-8.5). Bottled water, available commercially, was used for watering until the appearance of the first leaves. Five sets of pots were watered with five different concentrations of sodium cyanide. The sixth set was kept as control. The sodium cyanide concentrations used were 10, 20, 30, 40 and 50 mg/L. The watering scheme was such that a feed solution of 10 mL was applied to each pot once a week. 50 mL Commercial bottled water was used for watering on the other days. Throughout the study, three harvests were taken on differing dates. Root, stem and spike length, as well as wet and dry weights, were measured for each sampling. By taking the mean values of 100 individual plants and then of the three harvests, their statistical evaluations were carried out and the results applied with LSD test.

RESULTS AND DISCUSSION

For length measurements, *Triticum durum* roots showed a slight increase as compared with the controls. In stem height, however, a marked decrease was observed proportional to the amount of sodium cyanide applied (Table-1). In *T. aestivum*, root lengths in the 10 and 20 mg cyanide-treatments showed a slight increase compared with the control groups but in the other treatments with higher concentrations of sodium cyanide, the root length was proportional to the controls.

Spike lengths were also found to be proportional to the controls but the stems of all treated groups decreased in length (Table-2).

TABLE-1
EFFECT OF SODIUM CYANIDE ON PLANT HEIGHT OF *Triticum durum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root length (cm)	Stem height (cm)	Spike length (cm)
Control	18.55 ± 0.90	50.75 ± 1.22	3.92 ± 0.13*
10 mg cyanide	19.66 ± 0.23	48.34 ± 2.15	3.95 ± 0.19
20 mg cyanide	20.13 ± 1.54	50.30 ± 1.34	4.00 ± 0.18
30 mg cyanide	23.07 ± 1.26	45.13 ± 1.01	4.11 ± 0.14
40 mg cyanide	20.60 ± 1.30	49.23 ± 3.40	3.97 ± 0.12
50 mg cyanide	20.35 ± 1.97	44.32 ± 1.39	4.40 ± 0.15*

*Statistically significant ($p < 0.05$) difference in comparison to control medium.

TABLE-2
EFFECT OF SODIUM CYANIDE ON PLANT HEIGHT OF *Triticum aestivum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root length (cm)	Stem height (cm)	Spike length (cm)
Control	17.15 ± 0.85	63.05 ± 1.92	5.60 ± 0.21
10 mg cyanide	18.50 ± 1.13	58.25 ± 1.98	6.01 ± 0.24
20 mg cyanide	19.24 ± 1.04	58.25 ± 3.13	6.31 ± 0.45
30 mg cyanide	17.40 ± 0.80	59.36 ± 2.34	5.60 ± 0.17
40 mg cyanide	17.39 ± 0.86	60.68 ± 2.31	6.14 ± 0.33
50 mg cyanide	18.35 ± 0.87	62.12 ± 2.43	5.60 ± 0.28

When root fresh weights were examined, it was observed that while there was a decrease in the fresh weights of roots with 50 mg cyanide-treatment, the measurements of the other treated groups were equivalent to the control groups. However, there was an increase in stem fresh weight measurements for all the treated groups. A general decrease in the spike fresh weight was observed (Table-3).

In *T. aestivum*, while an increase was observed in the root, stem and spike fresh weights in plants with 10 mg and 20 mg cyanide-treatment, considerable decreases occurred at 30, 40 and 50 mg cyanide-treatments (Table-4).

In dry root weight measurements of *T. durum* plants, a decrease in the 50 mg cyanide-treated groups was observed and for the other application groups, the decrease is proportional to the control group. Dry stem weight showed a decrease in the 50 mg cyanide-treated group. Similarly, a decrease was also observed in the other treated groups. When spike weights were evaluated, there was a decrease in the 50 mg cyanide-treated group. However, in the other groups they were equivalent (Table-5). The decrease in grain weight is quite obvious (Table-7).

TABLE-3
ROOT, STEM AND SPIKE FRESH WEIGHTS OF *Triticum durum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root fresh weight (g)	Stem fresh weight (g)	Spike fresh weight (g)
Control	0.59 ± 0.07	1.28 ± 0.13	0.57 ± 0.06
10 mg cyanide	0.71 ± 0.01	1.43 ± 0.14	0.65 ± 0.09
20 mg cyanide	0.82 ± 0.11*	1.39 ± 0.11	0.56 ± 0.07*
30 mg cyanide	0.61 ± 0.09	1.31 ± 0.15	0.37 ± 0.05
40 mg cyanide	0.80 ± 0.12	1.57 ± 0.16	0.52 ± 0.09
50 mg cyanide	0.51 ± 0.06*	1.55 ± 0.12	0.33 ± 0.04*

*Statistically significant ($p < 0.05$) difference in comparison to control medium.

TABLE-4
ROOT, STEM AND SPIKE FRESH WEIGHTS OF *Triticum aestivum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root fresh weight (g)	Stem fresh weight (g)	Spike fresh weight (g)
Control	0.310 ± 0.055	1.330 ± 0.140	0.48 ± 0.14
10 mg cyanide	0.400 ± 0.05*	1.505 ± 0.125	0.66 ± 0.18
20 mg cyanide	0.285 ± 0.04*	1.590 ± 0.190	0.48 ± 0.34
30 mg cyanide	0.305 ± 0.03	1.155 ± 0.110	0.34 ± 0.13
40 mg cyanide	0.350 ± 0.06	1.375 ± 0.150	0.55 ± 0.21
50 mg cyanide	0.225 ± 0.03*	1.110 ± 0.120	0.43 ± 0.23

*Statistically significant ($p < 0.05$) difference in comparison to control medium.

In *T. aestivum*, the visibly increased wet weight measurements were not observed from the dry weight measurements. On the contrary, decreases in root, stem and spike weights of all treated groups were considerable as compared with the control groups. The increase in wet weights, which are not observed in dry weights, shows that the water content of plants with the 10 and 20 mg cyanide-treatments is higher than the others (Table-6).

There is a decrease in wet root weight proportional to the concentration of sodium cyanide used and a slight increase in dry spike weight. When grain weights were measured, it is apparent that the increases in cyanide concentration have a negative effect on plant productivity.

When transverse-sections were prepared from stems of *T. durum* seedlings and examined under light microscopy, it was discovered that there was a greater amount of stem sclerenchyma developed in plants treated with 50 mg sodium cyanide solutions. Transverse-sections were also taken

TABLE-5
ROOT, STEM AND SPIKE DRY WEIGHTS OF *Triticum durum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root dry weight (g)	Stem dry weight (g)	Spike dry weight (g)
Control	0.077 ± 0.10*	0.38 ± 0.03	0.03 ± 0.02*
10 mg cyanide	0.077 ± 0.01	0.40 ± 0.03	0.39 ± 0.04*
20 mg cyanide	0.080 ± 0.01	0.41 ± 0.03	0.37 ± 0.04
30 mg cyanide	0.087 ± 0.01	0.41 ± 0.03	0.30 ± 0.02
40 mg cyanide	0.090 ± 0.01	0.43 ± 0.03	0.36 ± 0.04
50 mg cyanide	0.060 ± 0.01*	0.39 ± 0.02	0.18 ± 0.01

*Statistically significant ($p < 0.05$) difference in comparison to control medium.

TABLE-6
ROOT, STEM AND SPIKE DRY WEIGHTS OF *Triticum aestivum* cv. PLANTS IN THE CONTROL AND APPLICATION GROUPS

	Root dry weight (g)	Stem dry weight (g)	Spike dry weight (g)
Control	0.340 ± 0.01*	0.49 ± 0.04*	0.50 ± 0.04*
10 mg cyanide	0.267 ± 0.01	0.48 ± 0.03	0.50 ± 0.05
20 mg cyanide	0.280 ± 0.01	0.48 ± 0.04	0.39 ± 0.06*
30 mg cyanide	0.260 ± 0.01	0.37 ± 0.03*	0.28 ± 0.03*
40 mg cyanide	0.240 ± 0.01	0.44 ± 0.03	0.39 ± 0.04*
50 mg cyanide	0.230 ± 0.01*	0.42 ± 0.04	0.35 ± 0.04*

*Statistically significant ($p < 0.05$) difference in comparison to control medium.

TABLE-7
WEIGHT OF GRAIN IN *Triticum durum* cv. AND *Triticum aestivum* cv. PLANTS

	Triticum durum cv.	*Triticum aestivum* cv.
Control	25.2817 ± 0.01	18.88 ± 0.01
10 mg cyanide	14.6977 ± 0.01	16.57 ± 0.01
20 mg cyanide	11.8832 ± 0.02	14.26 ± 0.01
30 mg cyanide	12.7394 ± 0.01	09.08 ± 0.01
40 mg cyanide	11.5679 ± 0.01	16.73 ± 0.01
50 mg cyanide	00.6690 ± 0.01	15.34 ± 0.01

from different parts of the leaves from both control and the 50 mg sodium cyanide treated groups. The leaf mesophyll was much less developed in plants of the latter.

Many of the important food plants human beings consume are cyanogenic. With an adequate amount of protein the human body is able to detoxify the amount of cyanide consumed. Cyanogenic plants are greatly preferred by farmers as they are very nutritive in their early stages and also have the ability to alienate herbivores. Cyanogenesis has obviously evolved as an effective chemical defence mechanism against herbivory. At least 262 (11 %) of 2378 species surveyed by Gibbs in 1974 were found to be cyanogenic[2]. As previously mentioned, the first 10 of 23 species grown in the highest amounts in the world are cyanogenic. These are cultivars of *Zea mays, Triticum aestivum/T. durum., Oryza* sp./*O. perennis, Hordeum vulgare*, *Manihot esculenta, Saccharum spontaneum, Phaseolus vulgaris/ P. lunatus, Sorghum bicolor, Avena sativa* and *Arachis hypogaea*.

In cyanogenic plants, the amount of HCN can vary on different parts of the same plant or different parts of the same species. For example, the HCN amount in Cassava varies from 240 to 890 mg/kg. In bulbs, this amount may be c. 1040 mg/kg and in chickpeas, 100-4000 mg/kg. The lowest amount is recorded in leaves of cereal crops with 6 mg/kg and highest amount in the panda's diet of bamboo shoots with c. 8000 mg/kg[3]. The concentrations given above are compared with the accepted acute lethal doses of HCN in mg/kg body weight of living organisms. These doses are 0.5-3.5 for humans[4], 2.4 for sheep[5], 2.0 for cattle[6], 3.7 for mice, 0.5-10.0 for rats, 2.0 for cats and 1.5 for dogs[7]. Acute cyanide poisoning accidentally caused by foods is certainly of frequent occurrence[3,8]. It is however, documented that leaves of cereals are cyanogenic but not their grains[9,10]. Nitrate can accumulate in drought-stressed barren corn plants but, cyanide toxicity from drought-stressed corn is rare[11].

When data from *T. aestivum* was examined, a considerable decrease in wet weight of 50 mg cyanide-treated individuals was observed. It is thought that although the treated plants have less water content than the control plants they are able to hold assimilation products formed by photosynthesis.

When *T. aestivum* and *T. durum* were compared as regarding stem height, in *T. aestivum* there was an increase in stem height, but in *T. durum* there was a decrease. Comparison of both species in terms of root, stem and spike dry weights showed a decrease in *T. aestivum*, parallel to the control group. The same decrease was observed in *T. durum*. The decrease in root, stem and spike weights as indicated in Table-3 is considerable. The wet weight measurements on the stems and the roots of *T. durum* reveal that there is an increase when treated with low sodium cyanide concentrations and a marked decrease when treated with 50 mg sodium cyanide. It is thought that this decrease is caused by the toxic effect of high concentrations.

According to the results of this study, the decrease proportional to cyanide concentration increase was remarkable for the last harvest when the plants have completed their development. The 108 % decrease in dry spike weight of 50 mg sodium cyanide treated plants of *T. aestivum* shows that the cyanide causes stress effects resulting in early spike development which bear, however, empty grain.

There was an increase in stem sclerenchyma and decrease in stem and leaf parenchyma in the treated groups as noted in transverse sections of stem and leaf.

The results obtained from this study indicate that with application of cyanide the plant reacts with decrease in stature and early grain formation. It additionally becomes infertile as seen from grain weight data. It is known that cyanogenic compounds can accumulate in vegetative parts or in seeds. It will be interesting to determine the cyanide content of various parts of the plant after treatment with sodium cyanide and to compare results obtained using cyanogenic and non-cyanogenic species.

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