RESEARCHES REGARDING THE INFLUENCE OF CERTAIN NATURAL HERBICIDES ON SEED GERMINATION

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Abstract: *The signaling and identification of phytotoxic substances in soil is a very difficult problem. The main cause of this difficulty is that many of these phytotoxic substances, especially the organic ones, are ephemeral, transform rapidly and this changes the character of their physiological action on plants. As germination is the main characteristic of sowing seeds, the phytotoxicity of extracts on the plant growth and development can be followed through the effects they produce on seed germination. A characteristic that can be followed is the way the radical system develops. The radicals in particular are especially sensitive to the action exerted by the phytotoxic substances. Thus, the region of the root apical meristem develops abnormally, exteriorizing by their discoloration and then their complete mortification. In most cases, the effect of the phytotoxic extracts on the apical meristem is very quick. The root necrosis is observed an hour after the exposure of the roots to the phytotoxic solution. The paper presents the possible phytotoxic effects on the seed germination that extracts from different indigenous plants and invertebrates could produce, as it is well known that these can be used to fight certain pests in the ecological agricultural crops. The experiment was done within the Phytotechny Laboratory of the Faculty for Natural and Agricultural Sciences – the Agriculture Department. Wheat seeds (Flamura 85 variety) were set to germinate, with aqueous extracts of celandine, wormwood, oily extract of Allium with 10 g sodium-free soap and extract of Coccinella diluted in ethanol. These extracts, used for the ecological fight against pests, were monitored to see if they have phytotoxic effect in the soil.*

Key words**:** *the phytotoxicity, phytotoxic extracts, the germination*

INTRODUCTION

In the recent years, the development of industry, transport, agriculture, forestry and urbanization have led sometimes to the pollution of the environment.

In these conditions, the environment must be protected against pollutants that lead to the degradation of water, air and soil.

The resulting products are very active and cause the chemical unbalance of any ecosystem. The primary sources of the pollutants that cause negative effects are:

- emissions resulted from coal burning, ore processing, refineries, liquid fuel burning, production of fertilizers and pesticides;
- industrial and domestic waste waters, muds resulted from the treatment of sewage waters, cinder from thermal power stations, industrial waste;
- chemicals used in agriculture, waste products and residues from the food industry, dejections from industrial animal farms.

The most known pollutants that accumulate in the superior layers of the soil are: Cd, Pb, Cu, Zn, Cr, As, Hg, Fe, F, Co, Se, Mn.

Plants are good indicators of the chemical makeup of soil. They will indicate the unbalance in the soil chemical composition caused by industrial or organic pollution, by changes in the chemical makeup or of the biochemical functions. There is an obvious confirmation regarding the correlation between the concentration of certain heavy metals in plant tissue and their accumulation in the soil. The relationship plant-soil is governed by a number of factors, among which the geochemical properties elements are very important.

Among the sources of soil pollution, a particular role is held by the use of chemicals

in agriculture. The increasing need for food has led to the use of methods to enhance the production per surface unit, and the use of chemicals for fertilization, weed, disease and pest control in cultivated plants are the most popular ones. The way these substances are used as well as the way they are made is often hazardous to the environment but also to human and animal heath.

The current selection of pesticides is evaluated at over 100 thousand chemicals based on about 1000 chemical compounds.

In the prevention and control of soil pollution, a particular role is held by the methodological aspects regarding the collection, keeping and preparation of soil samples that are to be examined, the analysis methods, the devices and equipment used to obtain exact information, according to the national legislation.

MATERIAL AND METHODS

The signaling and identification of the phytotoxic substances in the soil is a very difficult problem. The main cause of this difficulty is that many of these substances, especially the organic ones, are ephemeral, quick-changing and at the same time they change the type of their physiological action on the plants. Even though they are ephemeral, in their active stage they cause especially marking effects when they come in contact with the plant. This is why their presence must be followed in the dynamics of the biochemical processes that take place in the soil.

The most appropriate moment to identify organic phytotoxic substances is when they are produced in relatively large quantities. This moment corresponds with the production in large amounts of the radicular secretions, decomposition of organic waste from plants that produce phytotoxins themselves, with the period of maximal increase of phytotoxin-producing microorganisms, etc.

There are several methods to identify phytotoxins:

-biological methods, which include the study of the substances action on the microorganisms and plants susceptible to the action of the phytotoxins;

-chemical methods, which include qualitative or quantitative analyses with the help of reagents or paper/column chromatography;

Reiner and Nelson – Jones (quoted by KRASILNIKOV, 1958) claim that the phytotoxic substances are washed away by rain into the deeper layers of the soil in autumn and spring, while in winter, due to low temperatures, they become inactive. This leads to the diminished phytotoxic effect of certain substances that accumulate in the soil.

KRASILNIKOV (1958) thinks that the phytotoxic substances that accumulate in the soil are washed away by rain only partially and only those that were not absorbed by the soil colloids.

The phytotoxicity of the radicular, aqueous and alkaline extracts from the soil on the growth and development of plants can be followed through the effects they cause.

Thus, the effect of the extracts on the intensity of seed respiration can be seen. The greater the phytotoxicity of the extracts is, the more reduced the intensity of the seed respiration is.

Another feature that can be seen id the way in which the radicular system develops. The radices in particular are especially sensitive to the action of the phtotoxins. Thus, the region of the apical meristem of the roots develops abnormally, exteriorizing through its discoloration and then through its complete necrosis.

In most cases, the effect of the phytotoxic extracts on the apical meristem is very quick. The root necrosis can be observed an hour after the exposure of the roots to the phytotoxic solution under a microscope. An elongation of the root cells is observed and also a

cortical degenerescence. This effect can be used as diagnosis in proving the presence of the phytotoxic substances where there is a presumable phenomenon of soil exhaustion. The soil toxins can lead to the increase of plant cell permeability. The exudates of these cells have a high content of soluble sugars and substances that give positive reaction with ninhydrin.

MATERIAL AND METHODS

Germination is the main feature of the seeds destined for sowing. The phytotoxicity of the extracts on the growth and development of plants can be followed through the effects they cause on seed germination. A feature that can be traced is the way in which the root system develops. The radices in particular are sensitive to the action of phytotoxins. This effect can be used as diagnosis in proving the presence of the phytotoxic substances where they are supposed to be in the soil.

The purpose of the experience is observation of the possible phytotoxic effects the following extracts might cause on seed germination in the soil. It was executed in the Phytotechny Laboratory within the Faculty of Natural and Agricultural Sciences – The Agriculture Department. The germination was induced on autumn wheat seeds (Triticum aestivum vulgare), Flamura 85 variety, with aqueous extracts of celandine, wormwood and garlic (*Allium*) extract in oil with 10g sodium-free soap and Coccinella extract diluted in ethanol. The work versions were:

Witness version – germination in distilled water.
Version 1 – germination in aqueous extract of celandine as such, without water addition
Version 2 – germination in aqueous extract of celandine diluted in ½ water
Version 3 – germination in aqueous extract of celandine diluted in ¾ water
Version 4 – germination in aqueous extract of wormwood as such, without water addition
Version 5 – germination in aqueous extract of wormwood diluted in ½ water
Version 6 – germination in aqueous extract of wormwood diluted in ¾ water
Version 7 – germination in Allium extract in oil with 10g sodium-free soap as such, without water addition
Version 8 – germination in Allium extract in oil with 10g sodium-free soap diluted in ½ water
Version 9 – germination in Allium extract in oil with 10g sodium-free soap diluted in ¾ water
Version 10 – germination in Coccinella extract diluted in ethanol as such, without water addition
Version 11 – germination in Coccinella extract diluted in ethanol, diluted in ½ water
Version 12 – germination in Coccinella extract diluted in ethanol, diluted in ¾ water

For each version the experiment was repeated three times.

RESULTS AND DISCUSSIONS

The issue is to simulate in laboratory what will happen to the seeds in the field, if they will be affected by the ecological insecticide or not. The observations are presented in tables 1, 2, 3 and 4.

Table 1

The effect of natural insecticides extracts on the germination of wheat seeds

Crt. No.	Version	Germinated seed %				Normally developed embryos %
		R1	R2	R3	MEAN	
1	Witness version – germination in distilled water.	97	98	97	97.33	**100**
2	Version 1 – germination in aqueous extract of celandine as such, without water addition	97	97	97	97	100
3	Version 2 – germination in aqueous extract of	97	97	97	97	100

	celandine diluted in ½ water					
4	Version 3 – germination in aqueous extract of celandine diluted in ¾ water	97	97	97	97	100
5	Version 4 – germination in aqueous extract of wormwood as such, without water addition	96	97	96	96.33	100
6	Version 5 – germination in aqueous extract of wormwood diluted in ½ water	97	97	96	96.66	100
7	Version 6 – germination in aqueous extract of wormwood diluted in ¾ water	97	97	97	97	100
8	Version 7 – germination in Allium extract in oil with 10g sodium-free soap as such, without water addition	97	97	97	97	100
9	Version 8 – germination in Allium extract in oil with 10g sodium-free soap diluted in ½ water	97	97	97	97	100
10	Version 9 – germination in Allium extract in oil with 10g sodium-free soap diluted in ¾ water	97	97	97	97	100
11	Version 10 – germination in Coccinella extract diluted in ethanol as such, without water addition	96	97	96	96.33	100
12	Version 11 – germination in Coccinella extract diluted in ethanol, diluted in ½ water	97	97	97	97	100
13	Version 12 – germination in Coccinella extract diluted in ethanol, diluted in ¾ water	97	97	97	97	100

The conclusion is that all the embryos developed normally. There were no abnormally developed embryos in any of the versions or repetitions. Also, compared to the witness version, there were no significant differences in any of the versions or repetitions. Also, there was no increase of the germinative energy in any version or repetition compared to the witness in distilled water.

Germination of over 96% is considered normal (the state standards for sowing seed indicate over 85% germination). According to the experience results, we can say that the seeds are not affected negatively in the germination process in any of the versions or repetitions of insecticide extracts.

For more safety, germination was induced on wheat seeds of the same variety, Flamura 85 respectively, in the soil. The soil samples were collected from the plowed layer, from a depth of 0-20 cm, from typical worm chernozem from the "Dobrogea" Station for Agricultural Research and Development from Valu lui Traian. The seeds were set to germinate in 100g of soil in Petri dishes. The soil was first dried and sterilized in the drying closet. In order to create a proper environment for germination, the soil was brought to optimal germination humidity by moisturizing with distilled water.

Table 2

The effect of natural insecticides extracts on the germination of wheat seeds

Crt. No.	Version	Germinated seeds %	Difference compared to the witness	
			Difference	%
1	Witness version – germination in distilled water.	97.33	**MT**	**100**
2	Version 1 – germination in aqueous extract of celandine as such, without water addition	97	-0.33	99.66
3	Version 2 – germination in aqueous extract of celandine diluted in ½ water	97	-0.33	99.66
4	Version 3 – germination in aqueous extract of celandine diluted in ¾ water	97	-0.33	99.66
5	Version 4 – germination in aqueous extract of wormwood as such, without water addition	96.33	-1	98.97
6	Version 5 – germination in aqueous extract of	96.66	-0.67	99.31

	wormwood diluted in ½ water			
7	Version 6 – germination in aqueous extract of wormwood diluted in ¾ water	97	-0.33	99.66
8	Version 7 – germination in Allium extract in oil with 10g sodium-free soap as such, without water addition	97	-0.33	99.66
9	Version 8 – germination in Allium extract in oil with 10g sodium-free soap diluted in ½ water	97	-0.33	99.66
10	Version 9 – germination in Allium extract in oil with 10g sodium-free soap diluted in ¾ water	97	-0.33	99.66
11	Version 10 – germination in Coccinella extract diluted in ethanol as such, without water addition	96.33	-1	98.97
12	Version 11 – germination in Coccinella extract diluted in ethanol, diluted in ½ water	97	-0,33	99.66
13	Version 12 – germination in Coccinella extract diluted in ethanol, diluted in ¾ water	97	-0.33	99.66

The specialized literature shows that a feature that can be observed is the development of the root system. The radices in particular, are especially sensitive to the action of the phytotoxic substances. Thus, the region of the root apical meristem develops abnormally, exteriorizing through its discoloration and then through its complete necrosis. In most cases, the effect of the phytotoxic extracts on the apical meristem is very quick. The root necrosis can be observed an hour after the exposure of the roots to the phytotoxic solution under a microscope. An elongation of the root cells is observed and also a cortical degenerescence. This effect can be used as diagnosis in proving the presence of the phytotoxic substances where there is a presumable phenomenon of toxicity. In order to observe the influence of the natural insecticides on the development of the embryo roots in wheat, the following stages occurred: the seeds were set to germinate, first in distilled water till the appearance of three embryo roots; then they were treated on versions and repetitions with the solutions indicated above. Observations were effected an hour after the application of the solution on the seeds during germination, then two hours later, then three hours, twelve hours and twenty-four hours later. The conclusion was that an hour after treatment, in the witness version, the roots of the little wheat plants display a yellowish-ochre growing tip, while the rest of the root is translucent white, colors that are considered physiologically normal. No physiological modifications and no color differences were observed in the growing tip or the rest of the root in any of the versions. The situation remains constant after 2, 3 and 12 hours, respectively.

Table 3

The influence of the natural insecticides extracts on the grmination in soil of the wheat seeds

Crt. No.	Version	Observations
1	Witness version – germination in distilled water.	Normally developed embryos, normally developed plants
2	Version 1 – germination in aqueous extract of celandine as such, without water addition	Normally developed embryos, normally developed plants
3	Version 2 – germination in aqueous extract of celandine diluted in ½ water	Normally developed embryos, normally developed plants
4	Version 3 – germination in aqueous extract of celandine diluted in ¾ water	Normally developed embryos, normally developed plants
5	Version 4 – germination in aqueous extract of wormwood as such, without water addition	Normally developed embryos, normally developed plants
6	Version 5 – germination in aqueous	Normally developed embryos, normally developed plants

	extract of wormwood diluted in ½ water	
7	Version 6 – germination in aqueous extract of wormwood diluted in ¾ water	Normally developed embryos, normally developed plants
8	Version 7 – germination in Allium extract in oil with 10g sodium-free soap as such, without water addition	Normally developed embryos, normally developed plants
9	Version 8 – germination in Allium extract in oil with 10g sodium-free soap diluted in ½ water	Normally developed embryos, normally developed plants
10	Version 9 – germination in Allium extract in oil with 10g sodium-free soap diluted in ¾ water	Normally developed embryos, normally developed plants
11	Version 10 – germination in Coccinella extract diluted in ethanol as such, without water addition	Normally developed embryos, normally developed plants
12	Version 11 – germination in Coccinella extract diluted in ethanol, diluted in ½ water	Normally developed embryos, normally developed plants
13	Version 12 – germination in Coccinella extract diluted in ethanol, diluted in ¾ water	Normally developed embryos, normally developed plants

Observations effected 24 hours after contact with the natural insecticide extract are displayed in tables 4a, 4b, 4c.

Table 4a

The effect of celandine extract (Chelidonium majus L.) on the development of embryo roots in wheat

Crt. no.	Version	Image	**Observations**
1	Witness version – germination in distilled water.		The roots of the little wheat plants have ochre light-yellowish growing tip, translucent white on root length
2	Version 1 – germination in aqueous extract of celandine as such, without water addition		No necroses, brownish growing tip, yellow root length due to the natural pigments from the extract
3	Version 2 – germination in aqueous extract of celandine diluted in ½ water		No necroses, brownish growing tip, slightly yellow root length due to the natural pigments from the extract

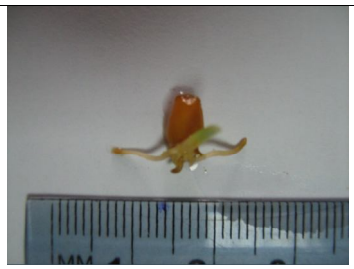
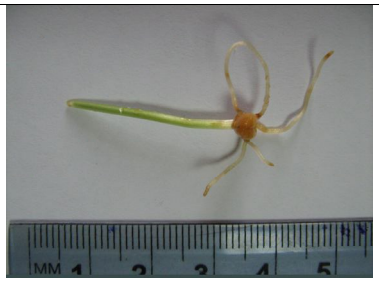
4	Version 3 – germination in aqueous extract of celandine diluted in ¾ water		No necroses, very light-brownish growing tip, slightly yellow root length due to the natural pigments from the extract

Table 4b

The effect of wormwood extract (*Artemisia absinthium* L.) on the development of embryo roots in wheat

Crt. No.	Version	Image	**Observations**
1	Witness version – germination in distilled water.		The roots of the little wheat plants have ochre light-yellowish growing tip, translucent white on root length
2	Version 4 – germination in aqueous extract of wormwood as such, without water addition		No necroses, yellowish-ochre growing tip, slighly yellow root length due to the natural pigments from the extract
3	Version 5 – germination in aqueous extract of wormwood diluted in ½ water		No necroses, yellowish-ochre growing tip, slighly yellow root length due to the natural pigments from the extract

4	Version 6 – germination in aqueous extract of wormwood diluted in ¾ water		The roots of the little wheat plants have ochre light-yellowish growing tip, yellowish-white root length

Table 4c

The effect of garlic extract (Allium sativum L.) on the development of embryo roots in wheat

Nr. Crt.	Version	Image	**Observations**
1	Witness version – germination in distilled water.		The roots of the little wheat plants have yellowish-ochre growing tip, translucent white on root length
2	Version 7 – germination in Allium extract in oil with 10g sodium-free soap as such, without water addition		The roots of the little wheat plants have yellowish-ochre growing tip, white root length and slightly lose their turgescence
3	Version 8 – germination in Allium extract in oil with 10g sodium-free soap diluted in ½ water		The roots of the little wheat plants have yellowish-ochre growing tip, white root length

4	Version 9 – germination in Allium extract in oil with 10g sodium-free soap diluted in ¾ water		The roots of the little wheat plants have yellowish-ochre growing tip, white root length

CONCLUSIONS

- The conclusion is that all the embryos developed normally. There were no abnormally developed embryos in any of the versions or repetitions. Also, compared to the witness version, there were no significant differences in any of the versions or repetitions. Also, there was no increase of the germinative energy in any version or repetition compared to the witness in distilled water.

- Germination of over 96% is considered normal (the state standards for sowing seed indicate over 85% germination). According to the experience results, we can say that the seeds are not affected negatively in the germination process in any of the versions or repetitions of insecticide extracts.

- The conclusion was that an hour after treatment, in the witness version, the roots of the little wheat plants display a yellowish-ochre growing tip, while the rest of the root is translucent white, colors that are considered physiologically normal. No physiological modifications and no color differences were observed in the growing tip or the rest of the root in any of the versions. The situation remains constant after 2, 3 and 12 hours, respectively.

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