Effect of Treatment with Saline Solution (NaCl) on Rape Plants in Presence of the Hemp Shives

SANDA COSARCA[1]*, IOANA ROSCA[1], ALINA COMAN[1], MONICA CRISTINA NAGY BOTA[2], CORNELIU TANASE[1]

[1]University of Medicine and Pharmacy of Tirgu Mures, Faculty of Pharmacy, 50 Gheorghe Marinescu Str., 540139, Tirgu Mures, Romania

[2] University of Medicine and Pharmacy of Tirgu Mures, Faculty of Medicine, 50 Gheorghe Marinescu Str., 540139, Tirgu Mures, Romania

The aim of this research was assessing the influence of hemp shives, like amendaments, in saline soils planted with rape (Brassica rapa L.). The growth and development assessing of rape plants in saline soils was achieved through biometric measurements for elongation and gravimetric measurements for the amount of biomass synthesized. Also, it was followed determination of the concentration of assimilating pigment (chlorophyll a, b, total carotenoid pigments) and protein content. It was found that adding hemp shives in soils with low salinity stimulated elongation and germination processes for rape. In soil with high salinity adding hemp shives determine an increase of biomass accumulation in all vegetative organs, comparative with the same salt concentration where we added the amendaments. Hemp shives can be recommended like natural amendaments for soils with high salt concentration.

Keywords: hampe shives, rape (Brassica rapa L.), saline soils, sodium chloride

In our century, the most important problem is the pollution. This represents the contamination of environmental with materials that interfere with human health, life quality or natural function of the ecosystems (living organisms and their environment). Most pollutants come from human activities [1-4]. Soil pollution concerns the accumulation of toxic chemicals, salts, pathogens or radioactive materials and heavy metals which may affect the life of plants, animals and humans [5-8]. For example, improper irrigation in areas where soil is not well drained, can have as result the accumulation of salt deposits that inhibits the growth of plants and can lead to sterility land [9-12]. In order to ensure the protection of soil, as a mean of increasing soil resources and environmental protection, different methods and technologies of remediation were designed to neutralize or block the flow of pollutants and to obtain an efficient and proper protection for a desired quality of soil [13,14]. In order to correct the alkaline reaction of soils it can be use various natural amendments [15]. Saline and alkaline soils have physical, chemical and biological properties that are unfavorable to the growth and development of plants. Salinity and alkaline reaction also cause changes in the processes of soil nutrient absorption by plants [16]. Plants have different capacities to support soil soluble salts: some are very sensitive, others are more resistant, and some of them, salt plants, are well adapted morphologically and physiologically to excess salts [17]. In order to determine the need to amend, saline and alkaline soils, the following are used: pH in aqueous suspension; the total soluble salt content, the sodium saturation of the adsorbent complex.

In some study which looked at the effect of salt stress on plants growth, it is show a connection between the decrease in plant length and the increase in the concentration of sodium chloride [18-24]. Other results showed the affection of leaf area negatively by using different concentrations of NaCl [23, 25, 26]. The influence of salinity on leaf number also increases with the increase in concentration [20, 27, 28]. The fresh and dry biomass of the shoot system are affected by changes in salinity concentration, type of salt present, or type of plant species [23, 24, 29-31].

The effect of sodium chloride (NaCl) concentrations on growth, chlorophyll content, protein content of rape (*Brassica rapa* L.) seedlings was investigated. The aim of this research was assessing the influence of hemp shives, like amendaments, in saline soils planted with rape (*Brassica rapa L.*).

Experimental part

Plant material

The experiments were carried out in a greenhouse, using (*Brassica rapa* L.) rape seeds obtained from Agrosem Impex S.R.L. Hemp shives used in this experiment are hemp waste resulting from fiber separation. At the time of hemp processing about 70-80% of the vegetal biomass amount is eliminated in the form of shives which are not conveniently used. However, their accessibility may lead to the use of polluted soils in bioremediation sequences.

Experimental plan. Intact rape seeds, which were homogeneous and identical in size and free from wrinkles, were sterilized with 10% Clorox for 5 min. We used a number of 10 growth vessels for each experimental variant. For one vessel, we used 100g of substrate, simple or with the hemp shives (1g / pot) where 5 rape seeds were sown. After sowing, 20 mL of NaCl solutions of different concentrations (table 1) were administered in the S1, S1h, S2, S2h, S3 and S3h variants. The rape seeds were left to grow in the greenhouse under natural lighting, (25/15)± 1°C (day/night) and 65% relative humidity. The pots were distributed randomly in lines, with each line comprising of all treatments. The obtained values were compared with those of the control, where saline solutions were not administered. A total of three replicates were chosen for each physiological measurement.

Growth measurements, for the plants exposed to saline treatments, were taken at 30 day after germination. The three replicates taken for each treatment were used to calculate the mean of each measurement. The measurements taken were elongation of the shoot system and stem, number of plant leaves, fresh weights of the plants.

* email: sandacosarca31@gmail.com

Photosynthetic pigments

0.05 g of fresh vegetal material was milled with quartz sand and extracted with acetone (80%). The carotenoids and chlorophyll content (chlorophyll *a* and chlorophyll *b*) were spectrophotometrically determined at specific wavelengths (470, 646 and 663 nm) and quantified using the equations (eq) 2 - 4:

chlorophyll a (μg/ mL) = 12.21 (A_{663}) - 2.81 (A_{646})

chlorophyll b (μg/ mL) = 20.31 (A_{646}) - 5.03 (A_{663})

carotenoids (μg/ mL) = (100 · A_{470} - 3.27 [chl a] - 104 [chl b])/ 22

where:

A_{663}, A_{646}, A_{470} represent the specific absorbance read spectrophotometrically; [*chl a*] and [*chl b*] are the *chlorophyll a* and *chlorophyll b* contents.

Extraction and determination of protein

0.5 g dry tissue were ground with10 ml filtrated water and then transferred quantitatively to test tubes. One millilitre of trichloroacetic acid (10%) was added, and then the tubes were placed in an ice bath for 15 min. The supernatant was separated from the precipitate and transferred to a centrifuge and run at 5000 rpm, for 15 min at 4°C. The precipitate was clarified in 20 mL sodium hydroxide (0.1 N) to dissolve the protein, and the volume was rounded up to the nearest whole number, in accordance with Lowry et al. (1951). Protein was determined using the Lowry method [32].

Results and disscusions

Effect of salt stress on plant growth

Analyzing the chart from figure 1, it can be noted that the value of germination energy and capacity decreases with increasing the concentration of NaCl applied on the soils. The probes that contain hemp shives reveals an increasing values of germination energy and capacity. Significant values are seen in the S1h variant, with a germination capacity of 100% compared to variant S1

Table 1

EXPERIMENTAL VARIANTS

Variants	*Type of soil*	*Soil g/pot*	*Hemp shives g/pot*	*NaCl – g/L*	*Number of pots*	*Number of seeds*
S0 – Control 1	non-saline soil	100	0	0	10	50
S0h – Control 2	non-saline soil + hemp shives	100	1	0	10	50
S1	slightly saline soil	100	0	4.2	10	50
S1h	slightly saline soil+ hemp shives	100	1	4.2	10	50
S2	moderately saline soil	100	0	8.4	10	50
S2h	moderately saline soil + hemp shives	100	1	8.4	10	50
S3	high saline soil	100	0	16.8	10	50
S3h	high saline soil+hemp shives	100	1	16.8	10	50

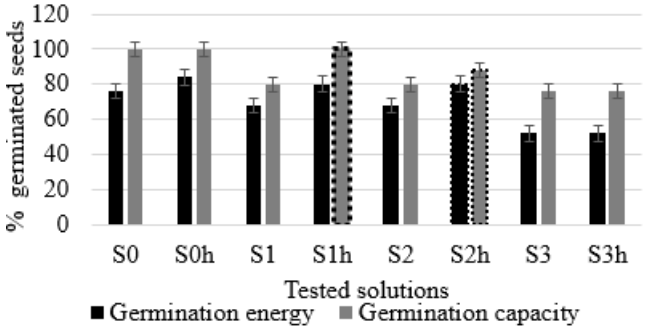

Fig. 1. Effect of treatment with NaCl in the presence of hampe shives on germination in rape seads

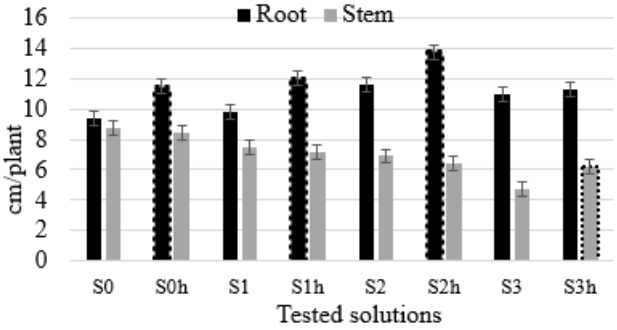

Fig. 2. Effect of treatment with NaCl in the presence of hampe shives on elongation process of rape root and stem

(without hemp shives) where the germination capacity was 80%. In high saline soils there are no significant differences in energy value and germination capacity.

Analyzing chart from figure 2, we find that the root system has developed better in the presence of saline solutions, with higher values in the variants where we added amendments. So, hemp shives added in saline soils determine better root system development. This result supports previous observations because it is known that the root system increases its absorption area in terms of water stress. By adding in the growth medium the saline solutions, the plants behaves like in a dry environment because of the osmotic difference.

Regarding the amount of biomass accumulation (fig. 3) in the stem and leaves results highlights the inhibitory effect of solutions NaCl with different concentrations. Adding amendments in polluted soils, the inhibitory effect is less pronounced. The total amount of accumulated biomass (root + strain + leaves) is increased in saline soils with hampe shives compared to those without hemp shives.

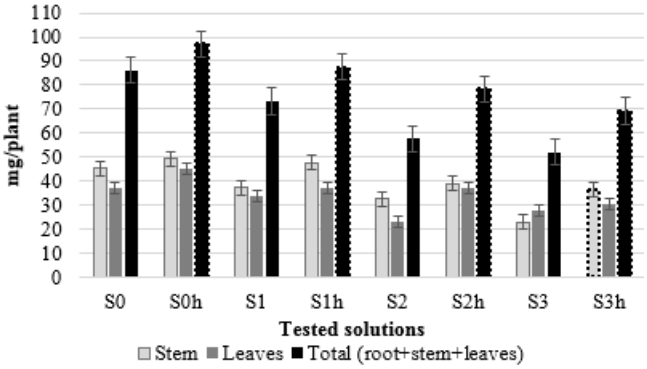

Fig. 3. Effect of treatment with NaCl in the presence of hampe shives on biomass acumulation process in rape plants

Effect of salt stress and hampe shives on photosynthetic pigments

Table 2 demonstrates the effect of saline solutions, using different concentrations of sodium chloride, on the chlorophyll content of the rape plants, including chlorophyll *a, b* and total chlorophyll. The results show an inverse relationship between salt concentration and chl. *a'* content. Whenever the concentration increased, chlorophyll *a* content decreased, reaching its lowest content compared to control plant. The amount of assimilating pigments synthesized by rape plants that were developed in the presence of hemp shives is slightly higher compared to the values obtained from the control (S0) or in the versions without hemp shives (S1, S2, S3). Significant values of total chlorophyll are observed in case of saline variants containing hampe shives.

Our results regarding a decrease in chlorophyll *a, b*, and total chlorophyll, agree with what Qados [23] reported, that the exposure of bean (*Vicia faba* L.) of sodium chloride led to the decrease in chlorophyll *a*, chlorophyll *b* and total chlorophyll content. The reduction of carotene content, are agree with results registered by Tort and Turkyilmaz [33], where they observed that the stressing of barley seedlings (*Hordeum vulgare* L.) with sodium chloride, reduced content of carotenes.

Effect of salt stress and hampe shives on protein content. In table 3 results indicate a positive effect of hampe shives in contact with sodium chloride on total protein of rape plant after 30 days. There was a general increase in protein content that corresponded with the increase in salt

Table 2
EFFECT OF TREATMENT WITH NaCl IN THE PRESENCE OF HAMPE SHIVES ON PHOTOSYNTHETIC PIGMENTS CONTENT (μg/g) FROM RAPE LEAVES PLANTS

	Chl a	Chl b	Carotenoids	Chl a+b	Chl a/b
S0	1106±4.42	291±1.41	200±1.21	1397	3.81
S0h	1242±6.24	311±2.14	222±1.41	1552	4.00
S1	1004±5.2	274±3.27	184±1.85	1278	3.67
S1h	1145±7.18	256±1.54	200±1.45	1401	4.47
S2	986±3.79	251±1.74	174±1.27	1237	3.94
S2h	1058±5.97	249±1.21	198±1.36	1307	4.25
S3	912±6.54	246±1.54	165±1.65	1158	3.71
S3h	986±5.45	256±1.11	197±1.44	1242	3.86

Values are mean of three replicates (n=3) ±SD

Table 3
EFFECT OF TREATMENT WITH NaCl IN THE PRESENCE OF HAMPE SHIVES ON PROTEIN CONTENT (mg/g) OF RAPE PLANTS

Variants	Protein concentration (mM) in root
S0	250.57±7.22
S0h	241.65±4.45
S1	259.88±5.13
S1h	258.43±2.54
S2	260.65±1.54
S2h	264.74±1.87
S3	278.74±1.44
S3h	277.65±1.68

Values are mean of three replicates (n=3) ±SD

concentrations. The results obtained, in general agree with other study [34-36]. Also, Tort and Turkyilmaz [33] recorded a big increase in protein content when treating barley plant (*Hordeum vulgare* L.) with 120 mM of sodium chloride. Kapoor and Srivastava [37] observed an increase in protein content in *Vigna mungo* (L.), when increasing salt concentration.

Conclusions

Hemp shives added to soils with low salinity favors the growth in length and hurries germination in the case of rapeseed plants. In enriched salinity environments the addition of hemp shives leads to an increase in biomass accumulation in all vegetative organs from rape compared to the same saline concentration environment in which no amendments have been added. It is argued that hemp shives can be natural amendments to soils with a high saline concentration.

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