

ORIGINAL RESEARCH PAPER

## ECOLOGICAL CONTRIBUTIONS TO SOME SOILS IN GREENHOUSES FOR VEGETABLE PRODUCTION

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Received: July, 09, 2021

Accepted: December, 03, 2021

**Abstract:** Due to the continuous growth of the population, there has always been a concern to increase vegetable production, but without the use of chemical fertilizers and pesticides. In this article, we propose a new direction for improving the soils of vegetable greenhouses, by using clays combined with certain substances to fulfil a protective role against pests and a role of enhancing the rooting of plants. For this experiment, we used hydrotalcite (anionic clay), to which we interspersed silver, and then we made a mixture with spirulina (*Spirulina Arthrospira Platensis*) and powder from willow extraction (*Salix L*). We worked comparatively on a soil that came from the soil harvested from Vanatori Neamt Natural Park, compared to a soil made in the laboratory by dosing this mixture (clays + spirulina + willow extract) in a proportion of 3, 5 and 7 %. The plant on which the experiment was performed was the green climbing bean *Phaseolus vulgaris L*, the “Theodora” biotype. The beans were purchased from the Research and Development Station for vegetable growing, Buzau. The experiment took place in a specially arranged greenhouse within the company FRUCTEX SRL from Bacau. Encouraging results were obtained regarding the germination rate of the beans, the height of the plants, the number of leaves and the number of pods.

**Keywords:** agriculture, beans, clay, nutrients, vegetables

## INTRODUCTION

Legumes are considered the second most important source of food after cereals [1]. Vegetables are very popular due to their low fat content and high protein content (20 to 45 %), essential amino acids, complex carbohydrates ( $\pm$  60 %), dietary fiber (5 to 37 %), minerals and essential vitamins. Legumes have also been called lean meat, and their incorporation into diets, especially in developing countries, could play a major role in eradicating malnutrition [2]. In addition to their nutritional properties, legumes are also assigned economic, cultural, physiological and medical roles due to their beneficial bioactive compounds [3 – 5]. Vegetable production largely depends on several factors, many of which are natural. The plant studied in this article is beans of the variety, *Phaseolus vulgaris* L., biotype “Teodora”.

It is part of the legume family that includes several agricultural plants that are also important food opportunities for the population. The first botanical description of the common bean was made by the botanists Tragus and Fuchs in 1542, under the name of “*Smilax hortensis*”. The generic name *Phaseolus* was introduced by Carl Linnaeus in 1753. The bean, or common bean (*Phaseolus vulgaris* L.), is a species of annual plant in the family *Fabaceae*, genus *Phaseolus*, according to the classification described by Chauv and Foury (1994) and Charles (1998). Both it and other food legumes play an important role in crop systems and in the diet of populations [6].

Beans are one of the most important seasonal vegetables and are widely grown around the world. Beans are rich in protein, carbohydrates, vitamins, minerals and dietary fibers [7] which contribute to balanced daily diets. Beans contain large amounts of phenolic acids, flavonoids and tannins [8] possess some bio-functionalities such as: anti-inflammatory, antidiabetic, antihypertensive, and antiproliferative [9]. Beans also contain some anti-nutritional compounds such as trypsin inhibitors, phytic acid,  $\alpha$ -galactosides, which can reduce the ability to digest and absorb its nutrients [10]. It also has important health benefits such as insulin stabilization, digestion regulation, brings an intake of vitamins A, B, E, but also minerals: iron, potassium. Beans have a high protein and fiber content and a low fat content [11 – 13]. The cultivation of beans is intended for human consumption (pods or seeds are eaten fresh or dried grains) and as feed: crop residues such as dried pods and stems [14 – 16].

The present study took place in a greenhouse where we had the opportunity to adjust the temperature, watering, soil pH within the company FRUCTEX SRL. Thus, the study focused on the germination and development of bean seeds from the “Teodora” variety, *Phaseolus vulgaris* L. “Teodora” which were purchased from the Research and Development Station for vegetable growing, Buzau. This climbing bean variety is a semi-early variety with dark green pods and is very suitable for planting in gardens.

In cultivation, germination of grains/seeds, this is a critical stage in the life cycle of higher plants [17 – 18].

That is why special attention was paid to this period of plant development, trying to meet the specific requirements of the biotype we worked with.

In the circumstances of the current vegetable growing in Romania and of the existing world trend, we want to contribute to the promotion of organic crops, which is a necessity for practicing a successful vegetable growing.

Because the development of the plants is directly influenced by the soil in which it grows, we propose the addition to the initial soil of a percentage of 10 % of anionic type

clays, modified by several additions. These additives refer to the creation of additional protection against harmful bacteria, but also to the creation of an environment conducive to the rooting and development of the plant. Spirulina and willow water by impregnation were added. These two constituents aim to influence the germination, rooting and development of plants, without using chemicals such as those in the auxin class.

## MATERIALS AND METHODS

The vegetable material was purchased from the Research and Development Station for vegetable growing, Buzau. The soil in which the beans were sown was brought from the Vanatori Neamt Natural Park. We considered that this soil is unpolluted and can be used for growing our plants.

To this soil, we contributed with the addition of modified anionic clays. Anionic clays were chosen, due to their special characteristics. These natural, or non-toxic materials have a two-dimensional structure in the form of parallel slats. Between the blades is an interlamellar space that can be greatly enlarged by intercalating water and various other substances of interest [19 – 22]. Taking advantage of this important characteristic of clays, we intervened, functionalizing them by adding AgNO<sub>3</sub>.

Spirulina, in powder form, was purchased from SC HOFIGAL EXPORT - IMPORT SA. We resorted to an addition of spirulina, due to the beneficial effect, described in the literature, on plant growth [23 – 28].

Growing vegetables generally requires the use of large amounts of chemical fertilizers and pesticides. They are expensive but can remain in residual concentrations in plants. Moving towards an ecological way of growing vegetables, it is normal to think about using green methods for their development. We turned our attention to biostimulators. Biostimulators are biological materials or extracts thereof having beneficial properties on soil, plant growth and development, increasing resistance to biotic and abiotic stress of plants. Among the existing biostimulators, algae are an important source of nutrients, growth regulators, fertilizers or pesticides [29 – 31].

Spirulina, which has as its scientific name *Spirulina Arthrospira Platensis*, is a filamentous cyanobacteria with a blue-green color. It is spiral in shape and is often considered an alga. The composition of spirulina varies depending on the conditions in which it is grown, the period and place of its harvest or the process of drying, crushing or packaging. It contains many essential nutrients for life: 70 % protein, 20 % carbohydrates, 5 % fat, 7 % minerals and 5 % water. There is a worldwide concern for the introduction of spirulina in various cultures, with the role of biostimulator and there is some research that suggests its importance in plant growth and development in a system of sustainable agriculture [32 – 35].

Another constituent introduced is the powder obtained by macerating willow (*Salix L.*) or weeping willow (*Salix babylonica*). The use of a willow macerate is used in many farms due to its antiseptic properties as well as its positive contribution to rooting [36 – 40]. The addition of willow water was the result of respecting a tradition gathered from farmers in the country and from small farmers. To obtain the extract, 100 grams of willow twigs cut to 1 cm were used, over which 1000 mL of distilled water was added at a temperature of 80 °C. The mixture was stirred for 24 hours at 400 rpm (rotations

per minute). During this time the constant temperature was maintained, achieving an extraction but also a concentration. To obtain the willow water powder, the concentration of the filtrate was continued at a temperature of 80 °C. In our laboratory, silver-doped clays were made. We considered, after consulting several data from the technical literature [41 – 45] that adding them will increase the resistance of plants to possible bacterial infestations, without the addition of other chemicals.

For the preparation of silver-doped clay, anionic clay was synthesized by the classical method, using precursors of aluminium and magnesium carbonate [46 – 48]. By establishing and adopting this method, a clay with special properties regarding the high specific surface was obtained to be able to intercalate the silver salt.

An amount of 0.5 grams of hydrocalcite type clay was introduced into a solution of 500 mL AgNO<sub>3</sub> of concentration 0.05 mol·L<sup>-1</sup>. The obtained mixture was stirred for 16 hours at a speed of 400 rpm and a temperature of 25 °C. Then the silver-doped clay material was filtered and dried [49, 50]. For a better reactivity of silver, it was reduced by adding 0.5 % formic aldehyde solution. The prepared material was then analyzed to see if a suitable spacing was obtained between the clay slats and a corresponding specific surface area.

A physical mixture of 80 % silver-doped anionic clay, 10 % spirulina powder and 10 % willow extract powder was made in the laboratory. This mixture was dosed in three experiments, namely:

- E1-Experiment 1 - soil from the forest to which we dosed 3 % the addition made in the laboratory (3 repetitions);
- E2-Experiment 2 - forest soil with an addition of 5 % (3 repetitions);
- E3-Experiment 3 - forest soil with the addition of 7 % mixture of clay, spirulina and willow extract (3 repetitions);
- EM-Experiment M - forest soil, without additives, considered as a control sample, (3 repetitions).

The pots were filled with the respective soils, and in each pot 2 beans were planted. As a control sample, the soil harvested from Vanatori Neamt Natural Park, from a depth of 15 cm was considered. The development of bean seeds from planting to a duration of 10 weeks was studied. This period was considered optimal, because it was possible to see the germination and development of the plant until the appearance of bean pods, inclusive. All pots were kept in the greenhouse, in optimal conditions of temperature, humidity and light.

During the experiment, analyses were performed, namely: analysis of clays, soil and measurement of plant development. The chemical analysis was performed with the contribution and help of the Research-Development Station for Vegetable Culture, Buzau and the National Research and Development Institute for Industrial Ecology (ECOIND) Bucharest. In the greenhouse, measurements were made on the development of beans planted at 6, 8 and 10 weeks.

## RESULTS AND DISCUSSIONS

In the experiment it was used as a witness, a soil that comes from the forest, from the Vanatori Neamt Natural Park. The analysis performed on this soil is presented in the Table 1.

Table 1. Soil characteristics

Density apparent [g·cm <sup>-3</sup> ]	Ca [mg·Kg <sup>-1</sup> soil]	Mg [mg·Kg <sup>-1</sup> soil]	K [mg·Kg <sup>-1</sup> soil]	Na [mg·Kg <sup>-1</sup> soil]	P assimilable [mgP <sub>2</sub> O <sub>5</sub> /Kg soil]	C organic [%]	Organic matter [%]	N total [%]	pH
1.19	499	425	2.98	0.15	3.9	7.99	14.03	0.28	6.4

To perform soil analysis, soil samples were collected from the forest from a depth of 15 cm. From the obtained results, it results that we can use this soil as a control sample to estimate the evolution of the planted beans [51 – 55]. The amount of organic carbon and N, the presence of other nutrients and slightly acidic pH, demonstrate that the soil can be used successfully as a culture medium without the need to add fertilizers.

For the comparative study we proposed, we also used silver-doped anionic clays. The characterization of the clays is shown in Figures 1, 2 and 3.

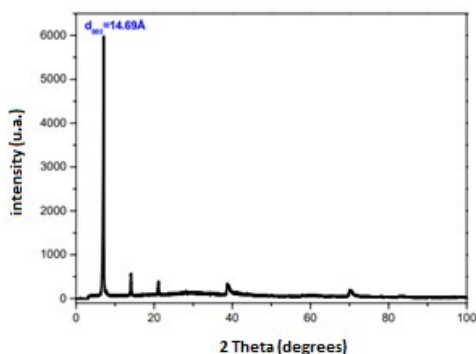


Figure 1. Prepared anionic clay diffractogram

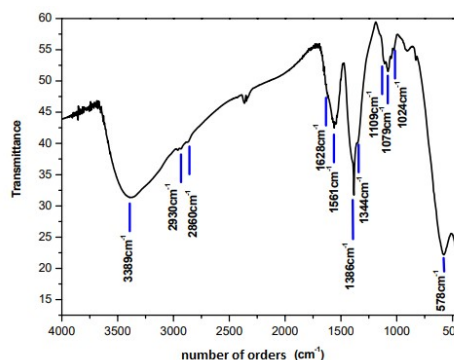


Figure 2. FTIR spectrum of prepared anionic clay

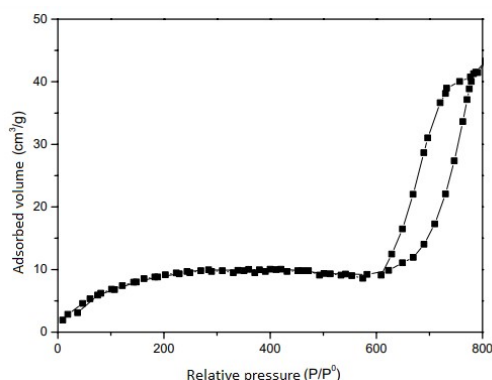


Figure 3. Adsorption / desorption isotherm determined by BET method

The diffractogram in Figure 1 has a specific appearance for anionic clays. This analysis was performed at ECOIND using a Siemens D501 diffractometer. It is important to be able to see the distance between the slats of anionic clay. In the diffractogram you can distinguish several areas. The first area is specific to small angles and has very strong reflections, which help us to find the basal distance and hence the distance between the layers. This distance is an important property for prepared clays. In our case, we

obtained an interlamellar distance of 14.67 Å, which is a very good distance. It depends a lot on the anion (salt) from which we started and the degree of swelling, enlargement of the interlamellar space, due to soaking with water. The asymmetry of the peaks in the diffractogram is explained by the existence of a degree of disorder in the clay structure. Not all slats are placed parallel to each other. This is due to a deficiency in preparation, which in our case is not very important, the clay being then mixed with other components, without a claim that the material respects a crystallinity. In the diagram in Figure 2, there are also adsorption bands corresponding to CO<sub>3</sub> anions, from which the anionic clays were synthesized (1561 - 1344 cm<sup>-1</sup>). Adsorption bands with lower values are characteristic of vibrations of Me-O bonds and deformation of Me-O-Me bonds. This analysis was performed at ECOIND on a FT-IR device type Spectrum BX II Perkin Elmer, to identify the molecular groups that are present in the prepared clays: anions, molecular groups and water molecules.

Another important analysis, through which we can characterize the prepared clays, is the determination of the adsorption / desorption isotherms by the B.E.T. (Table 2). The analysis was performed on the Quantracrom UK Limited device, Autosorb 1C series. We considered it important to identify the texture of the prepared porous media. This is done by adsorption - physical desorption of nitrogen (N<sub>2</sub>) at its liquefaction temperature (77K). The adsorption / desorption isotherm of the prepared clay is shown in the Figure 3.

*Table 2. Morphological characteristics of clay*

Prepared clay	S BET [m <sup>2</sup> ·g <sup>-1</sup> ]	The volume of a layer Vs [cm <sup>3</sup> ·g <sup>-1</sup> ]
	63.37	7.8

From Figure 3, it can be seen that the adsorption / desorption isotherms show an H3 type hysteresis. It results that the clay material prepared in the laboratory, has a mesoporous structure with intergranular pores, which develops a specific surface of 63.37 m<sup>2</sup>·g<sup>-1</sup>.

The existence of silver in clays was verified by atomic adsorption with the Qatomoca Spectrophotometer, SOLAR M6 DUAL.

By characterizing the clays prepared in the laboratory, we demonstrated that they have a very good specific surface, able to soak with water and then eliminate the water in the culture medium gradually, to promote the development and germination of plants.

### Results on the study of greenhouse plant development

After planting the beans, we followed the development of the seeds and their transformation into plants. A temperature around 20 °C was maintained in the greenhouse and an appropriate watering regime was practiced. The beans germinated and it was seen with the naked eye that they were rising from the ground after 8 days.

In the control pots (EM), 6 beans were planted (2 beans / pots x 3 repetitions). In these, 5 plants emerged, achieving a proportion of 83.3 %. In the pots that form Experiment no. 1 (E1) Experiment 2 (E2) and Experiment 3 (E3), all plants emerged. So germination took place 100 % in pots. At 6 weeks the development situation of bean plants is presented in the Table 3.



**Table 3.** Number of leaves, height and number of flowers and pods after 6 weeks

Experiment	No. leaves						Ave rage	Plant height						Ave rage
EM	15	15	16	15	17	15	15	32	30	31	32	32	32	31
E1	20	21	20	21	22	20	20	35	34	34	35	35	35	35
E2	24	25	26	26	27	25	25	38	39	38	39	39	39	39
E3	26	27	28	28	28	27	27	39	40	40	41	40	42	40

After a period of 8 weeks, plant development is shown in Tables 4 and 5.

**Table 4.** Number of leaves and plant height after 8 weeks

Experiment	No. leaves						Ave rage	Plant height						Ave rage
EM	38	37	37	39	38	40	38	126	125	120	129	127	124	125
E1	42	41	41	43	42	44	42	129	128	129	133	131	130	130
E2	46	47	45	48	47	49	47	133	132	129	137	136	135	134
E3	47	48	46	49	49	50	48	134	133	131	137	137	136	135

**Table 5.** Number of flowers and number of pods after 8 weeks

Experiment	No. flowers						Ave rage	No. of pods						Ave rage
EM	3	4	4	3	2	4	3	2	3	2	2	2	3	2
E1	6	7	7	6	6	7	7	6	7	7	6	6	7	7
E2	10	11	12	11	10	12	11	10	11	12	11	10	12	11
E3	11	12	14	12	11	14	12	11	12	14	12	11	14	12

Tables 6 and 7 show the evolution of bean plants, measured at 10 weeks.

**Table 6.** Number of leaves and plant height after 10 weeks

Experiment	No. leaves						Ave rage	Plant height						Ave rage
EM	60	59	58	62	58	64	60	220	219	219	228	222	217	221
E1	64	64	63	66	66	68	65	223	222	223	231	225	230	226
E2	68	67	66	70	67	72	68	228	227	228	236	230	234	230
E3	69	68	67	71	69	73	69	229	228	229	237	231	235	231

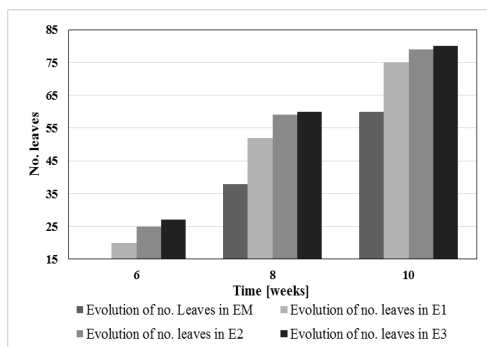
**Table 7.** Number of flowers and number of pods after 10 weeks

Experiment	No. flowers						Ave rage	No. of pods						Ave rage
EM	5	7	8	5	4	5	5	10	12	14	10	9	12	11
E1	9	10	11	8	8	9	9	13	15	17	13	13	15	14
E2	14	14	15	13	14	14	14	18	20	22	18	18	20	19
E3	15	16	16	14	15	16	15	19	21	23	19	19	21	20

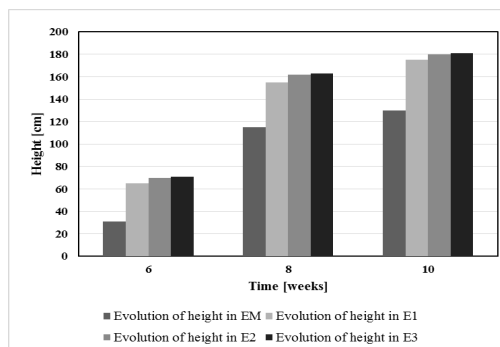
The tables show that measurements were made regarding no. of leaves, height, no. of flowers and pods.

The growing conditions of the plants were kept constant, due to the fact that the experiments were done in a high-performance greenhouse.

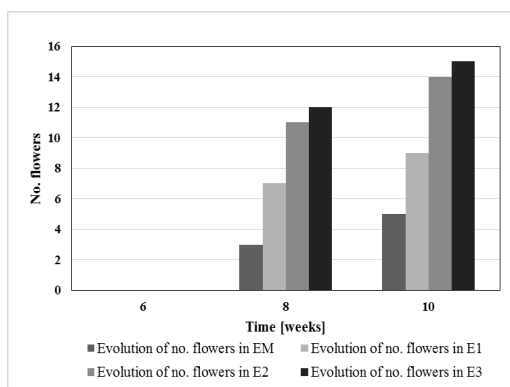
The development of bean plants, in the experiments, is shown in the Figures 4 -7.



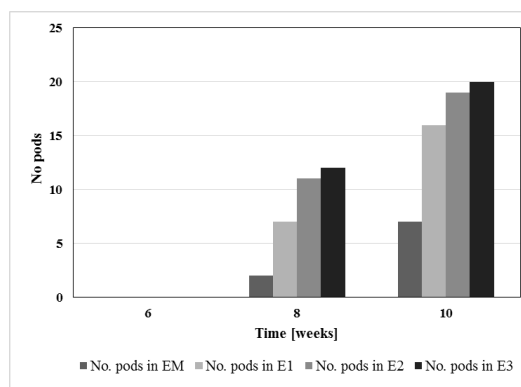
**Figure 4.** Evolution of no leaves in experiments, measured at 6, 8 and 10 weeks



**Figure 5.** Evolution of plant height in experiments, measured at 6, 8 and 10 weeks



**Figure 6.** Evolution of the number of flowers of the plant, in experiments, measured at 6, 8 and 10 weeks



**Figure 7.** Evolution of the number of plant pods, in experiments, measured at 6, 8 and 10 weeks

As can be seen from Tables 3 - 7 and Figures 4 - 7, the bean plants had a very good development and made flowers and pods.

As can be seen from Figures 4, 5, 6 and 7, the beans grew very well even in the control sample (EM), because the soil in which the beans were planted was a good soil (Table 1), which allowed a normal development of the plants. But through experiments E1, E2 and E3, we wanted to find out if by intervening with a small percentage of natural substances mixture (from 3 to 7 %), we can get a better production of bean pods. The results we obtained were encouraging.

Regarding the response of bean plants to treatments applied, the amounts of clay, spirulina and willow water had very noteworthy on improving the parameters of growth and development measured in beans plants. The cultivation of beans requires important quantities of chemical fertilizers and pesticides, which can impair the nutritional quality of the fruits, produced and be harmful to the environment.



## CONCLUSIONS

This study aimed at determining the biostimulant effects of clays doped with silver, of Spirulina and willow extract on the growth and development of beans without the use of chemicals.

From the experiments performed, it can be said that there were positive effects following the introduction of additives in the soil in the greenhouse. We noticed increases in the number of leaves, an increase in the height of the plants and especially an increase in the number of flowers and pods. The addition of clay facilitated the maintenance of moisture in the plants for a longer time and its gradual release. But the clay was doped with silver, which greatly increased its properties [56 – 58]. In the experiments, Ag behaved as an antibacterial agent, which induced a state of health in plants throughout development: from germination to the appearance of bean pods. Spirulina and willow extract behaved like auxins, but without auxins but natural substances. The addition of the mixture of spirulina, willow extract, along with silver-doped clays, had a beneficial effect on the development of beans.

In the present study, we were interested in the effect of this mixture added to the soil harvested from the forest, in the development of beans. The best results were obtained in the E2 experiment, where they had an addition of 5 % to the initial soil. Between experiments E2 and E3, a small difference in growth was obtained and we consider that it does not justify an increase in dosage from 5 to 7 %.

Also, we wanted to show that we can get a good production of bean pods without using fertilizers, pesticides and growth hormones. But by using certain natural materials, we can have very good productions.

The mixture consisting of the three components clay doped with silver, spirulina and willow water, we consider that it had a protective role against possible pests, helped the rooting and development of plants.

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