

## EFFICIENCY OF PRE-SOWING TREATMENT OF ECHINACEA PALE SEEDS ON MORPHOMETRIC INDICATORS OF SECOND YEAR OF VEGETATION PLANTS

### ЕФЕКТИВНІСТЬ ПЕРЕДПОСІВНОЇ ОБРОБКИ НАСІННЯ ЕХІНАЦЕЇ БЛІДОЇ НА МОРФОМЕТРИЧНІ ПОКАЗНИКИ РОСЛИН ДРУГОГО РОКУ ВЕГЕТАЦІЇ

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#### ABSTRACT

*The morphometry of echinacea pale (Echinacea pallida) plants grown from seeds treated with environmentally friendly growth stimulants was studied. It is proved that pre-sowing treatment of seeds is manifested in the differences of morphometric features that determine the yield of this crop. It is determined that the greatest stimulating effect on the height and weight of stems, leaf weight has pre-treatment of seeds with Nanomix fertilizer or UHF irradiation. The nature of the effect of stimulants on stem height, weight and number of leaves is similar between years, and the effect on stem weight has annual dependency.*

#### АННОТАЦІЯ

*Досліджено морфометрію рослин ехінацеї блідої (Echinacea pallida), що були вирощені з насіння обробленого екологічно безпечними стимуляторами росту. Показано, що передпосівна обробка насіння ехінацеї проявляється у відмінностях морфометричних ознак, які обумовлюють урожайність цієї культури. Визначено, що найсильніший стимулюючий ефект на висоту та масу стебел, масу листків має передпосівна обробка насіння наноміксом або УВЧ-опроміненням. Особливості впливу стимуляторів на висоту стебел, масу та кількість листків подібні між роками, а вплив на масу стебел має річні залежності.*

#### INTRODUCTION

Medicinal crop production is one of the important areas of agriculture. Areas for growing medicinal raw materials in Ukraine are increasing every year. Preparations based on species of the genus Echinacea are among the most popular herbal supplements in Europe and North America since the 1980s, because they stimulate the immune system and are used to prevent or treat colds (Barrett et al., 2010; Woelkart et al., 2008). Laboratory and clinical studies have yielded mixed results on the efficacy of such drugs (Barrett et al., 2010; Ross, 2010; Md. M. Billah 2018; Woelkart et al., 2008), but they have taken a significant place in the market and this trend is most likely to continue (Barrett et al., 2010; Qu, Widrlechner, 2012). Three species of Echinacea (E. purpurea, E. angustifolia, E. pallida) have medicinal value (Dobrange et al., 2019) and are commercially sold as medicinal plants.

Compared to E. angustifolia or E. purpurea, E. pallida is less common for dietary supplements, but studies of E. pallida indicate potential medical value, including anti-cancer (Chicca et al., 2007), antiviral (Schneider et al., 2010), anti-inflammatory (Qu, Widrlechner, 2012) and accelerating wound healing. Significant depth of dormancy of seeds is also characteristic of E. pallida and the effects that are effective for dormancy of E. angustifolia are also effective for E. pallida (Qu et al., 2004; Mahmood Attarzadeha et al 2020).

A significant share of crop production (about 25-30%) is lost due to poor quality sowing seeds. More than 30% of the seed is unsuitable for sowing due to low germination and insufficient germination energy. A partial solution to this problem is the introduction of modern, cost-effective, energy-saving, environmentally friendly technologies for pre-sowing seed stimulation (Petrovsky, 2014).

For companies specializing in the cultivation of medicinal plants, obtaining simultaneous germination of echinacea remains an important economic problem, because, despite the high laboratory germination (from 50 to 95% depending on the species), in the field it is much lower and unstable over the years (Pospelov, 2013). Although Echinacea plants can be grown vegetatively (Feghahati, Reese, 1994), production in the field is done by seed sowing. To stimulate seed germination, stratification, synchronization techniques (mechanical,

chemical), gibberellin, ethephon, sodium nitrate, nitric acid, ethanol, alternation of dark and light time, temperature for interruption of rest are used (Qu et al., 2004).

The problem of the influence of ecologically safe seed growth stimulators on the dynamics of *E. pallida* ontogenesis remains unexplored. The height of plants, as well as their foliage and total leaf surface area, are important morphological features in the cultivation of crops (Shevel, 2017). It depends on the characteristics of the variety, the level of moisture, the background of mineral nutrition, air temperature and other environmental factors. Analysis of stem height makes it possible to determine the most optimal conditions for the formation of highly productive agrophytocenoses (Varavva, 2007).

The height of plants during the harvest period indicates not only the influence of weather conditions, but also to some extent reflects the level of agricultural techniques of crop production. When applying the morphometric approach, any feature in its quantitative expression acts as a parameter, and the plant as a whole or its individual parts are characterized by a set of such parameters (Zhukov, 2011, 2015, 2017). Morphometric parameters are divided into two groups: meristic - counting, with a unit of measurement piece, and metric - are measured in units of mass, length, surface, volume, and others. In some cases, they are accompanied by features that characterize the spatial position of the plant or its parts (Zlobin et al, 2009).

The aim of our work was to study the differences of morphometric features of *Echinacea* pale plants grown from seeds that have been treated with environmentally friendly growth stimulants.

## MATERIAL AND METHOD

In 2012, 2014 and 2015, the seeds of *Echinacea pallida* (Nutt.) Nutt of the Krasunia Preriy variety were harvested from the 2011, 2013 and 2014 harvest, respectively. For the analysis of indicators of productivity of an echinacea pale the plants of the second year of vegetation were taken.

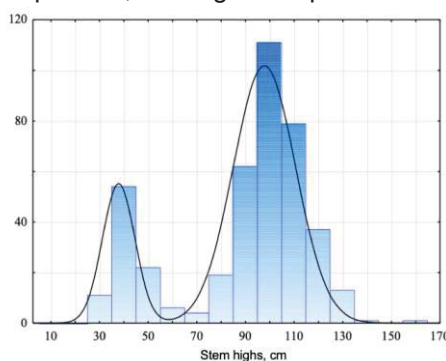
The works were carried out on the industrial plantations of *Echinacea* pale in the conditions of Poltava region, Ukraine. The soils are represented by black soils of medium granulometric composition with a humus content of 2.45–2.84%, aqueous pH 6.4. The previous crop was winter wheat. Seeds were sown with a SST-12B seeder with a sowing rate of 8–10 kg / ha. Row spacing - 45 cm. During the growing season, inter-row tillage, plant fertilization and manual weeding were carried out. For field studies after comparison in the laboratory, the following seed treatments were taken: control - dry seeds; treatment of echinacea seeds with electromagnetic field of the UHF range (UHF irradiation); soaking the seeds in a 0.001% mix of Potassium humate (humate); soaking the seeds in a mixture of 0.001% of sodium humate and 1% mix of chelated complex fertilizer "Nanomix" (humate + nanomix); soaking the seeds in a 1% mix of chelated complex fertilizer "Nanomix" (nanomix).

Treatment with chemicals of natural origin was carried out by soaking the seeds in an aqueous mixtures of preparations. To treat seeds with UHF radiation, we used a UHF 60-Med TeCo device, which uses a frequency of 27.17 MHz, has an output power of up to 60 W, allowed for use by the Ministry of Health of Ukraine. The size of one batch of irradiated seeds - 3 g. For irradiation, plates with a diameter of 0.12 m were connected to the UHF generator. The distance between the plates was 0.05 m. After irradiation, the number of seeds required for the experiment was selected from the batch.

Statistical analysis was performed using the program Statistica 10.0.

## RESULTS

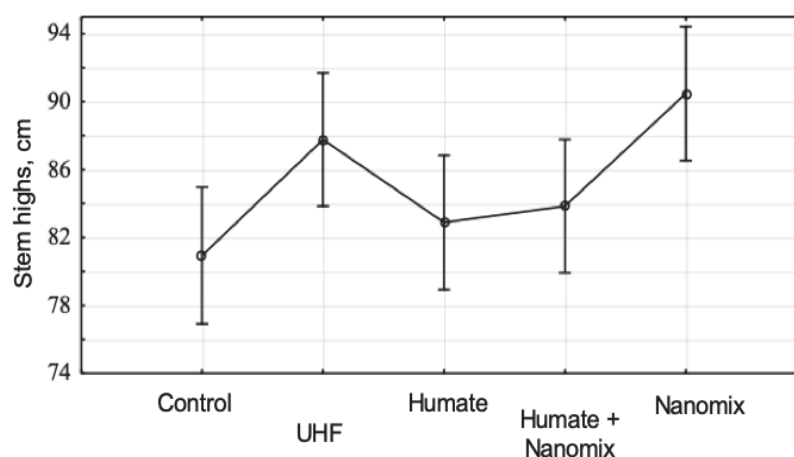
An important marker of the production process is the height of the stems. The histogram of the distribution of this indicator has a clear bimodal distribution (Fig. 1). The model of a mixture of two Gaussian distributions well describes the data on the height of the stems of *Echinacea* pale (Kolmogorov-Smirnov statistics  $d = 0.05$ ;  $p = 0.23$ ). The smaller component of the mixture is characterized by an average value of  $37.83 \pm 6.68$  cm and is 21.99% of the sample size, the larger component -  $97.91 \pm 12.84$  cm (78.01%).



**Fig. 1 - Histogram of the distribution of values of the echinacea stems height**

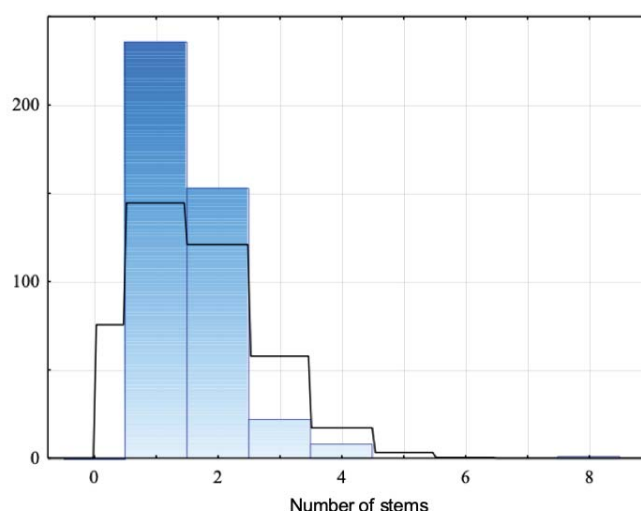
Within the general linear model, the variability between years and the effect of pre - sowing seed treatment determine 55% of the variation in the height of the stems of Echinacea pale during the study period ( $R^2 = 0.55$ ;  $F = 38.51$ ;  $p = 0.00$ ). Interannual features and method of seed treatment are statistically significant predictors. The relationship between these indicators is not a statistically significant predictor, indicating that the nature of the response of stem height to seed pre-treatment does not change between years.

In the control version, the height of the stems is  $80.94 \pm 2.05$  cm (Fig. 2). Pre-treatment of seeds with Nanomix has the greatest stimulating effect on stem height. In this case, the height of the stems of Echinacea pale reaches the level of  $90.48 \pm 2.01$  cm. Slightly less stimulating effect has UHF irradiation of seeds -  $87.77 \pm 1.99$  cm. Humate and a mixture of humate and nanomix are showing less effectiveness on the height of the stems -  $82.88 \pm 2.01$  and  $83.85 \pm 1.99$  cm, respectively. The highest level of stem height was recorded in 2013, and the lowest - in 2015. The nature of the impact of stimulants on stem height is similar between years. But it should be noted that in 2016 the effectiveness of the impact was the lowest.



**Fig. 2 - Variation in the height of the stems of Echinacea pale depending on the type of pre-sowing seed treatment: abscissa - methods of pre-sowing seed treatment, ordinate - the value of the stems height due to the influence of pre-sowing treatment**

The number of stems of Echinacea pallida varies from 1 to 8 with a median value of 1. The distribution of the number of stems deviates to some extent from the binomial distribution due to the predominance of plants with 1 or 2 stems and a reduced proportion compared to random alternative plants with 3 or more the number of stems (Fig. 3).



**Fig. 3 - Histogram of the distribution of the number of stems. Indicator line points to a hypothetical binomial distribution**

The number of stems is somewhat invariant in the sense that only 7% of its variation can be explained by predictors such as year and method of pre-sowing seed treatment ( $R^2 = 0.07$ ;  $F = 3.41$ ;  $p = 0.00$ ). This

conclusion is confirmed by the fact that statistically significant predictors of the number of stems are a year and a constant equal to  $1.52 \pm 0.04$  pcs.

We found statistically significant annual levels of stems (Fig. 4). The largest number of stems of *Echinacea pale* was characteristic of the 2013 harvest and was  $1.73 \pm 0.06$ . In 2015, this indicator decreased to the level of  $1.62 \pm 0.06$ . The smallest number of stems was characteristic of the 2016 harvest and was  $1.21 \pm 0.07$ . Pre-sowing treatment of seeds has no effect on the number of stems of *Echinacea pale* during harvest.

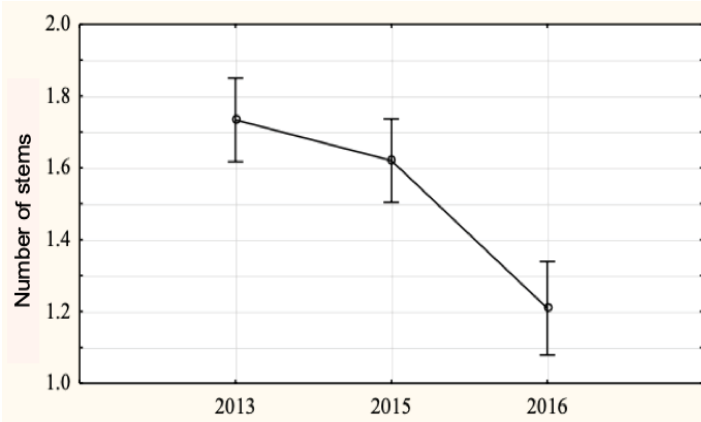


Fig. 4 - Variation in the weight of the stems of *Echinacea pale* depending on the year of harvest

The weight of echinacea stalks pale during harvest varies in the range from 2.46 to 31.33 g with an average value of  $14.91 \pm 0.26$  g. The weight of stems correlates with their height ( $r = 0.34$ ,  $p = 0.00$ ) and quantity ( $r = 0.33$ ,  $p = 0.00$ ). The distribution of the mass of stems is well described by the normal law (Fig. 5). The type of seed treatment before sowing and interannual features can explain 53% of the variation in stem weight ( $R^2 = 0.53$ ;  $F = 34.83$ ;  $p = 0.00$ ).

All predictors and their relationship are statistically significant. In the control, the weight of the stems is  $14.58 \pm 0.41$  g. The plants differed insignificantly from the control after pre-treatment of seeds with UHF irradiation and a mixture of humate and Nanomix ( $15.29 \pm 0.40$  and  $14.37 \pm 0.40$  g, respectively). The highest value of stem mass was found for plants grown from seeds pre-treated with Nanomix (Fig. 5). This figure reached the level of  $16.42 \pm 0.41$  g. Pre-treatment of seeds with humate had the effect of reducing the weight of the stems, which in this case weighed  $12.63 \pm 0.41$  g.

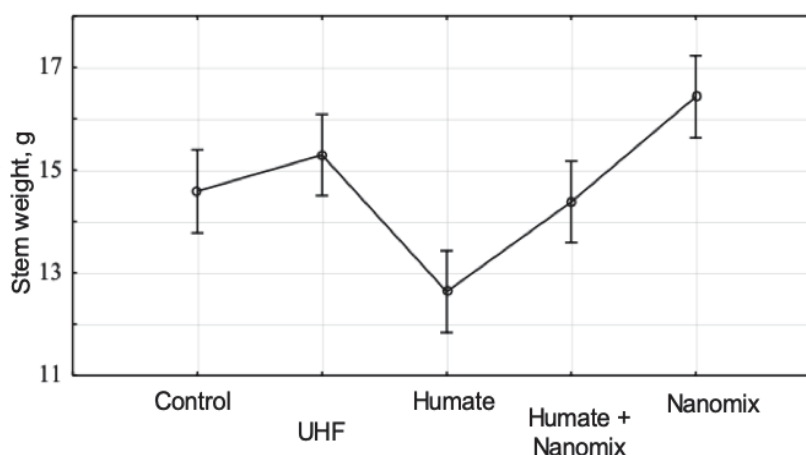
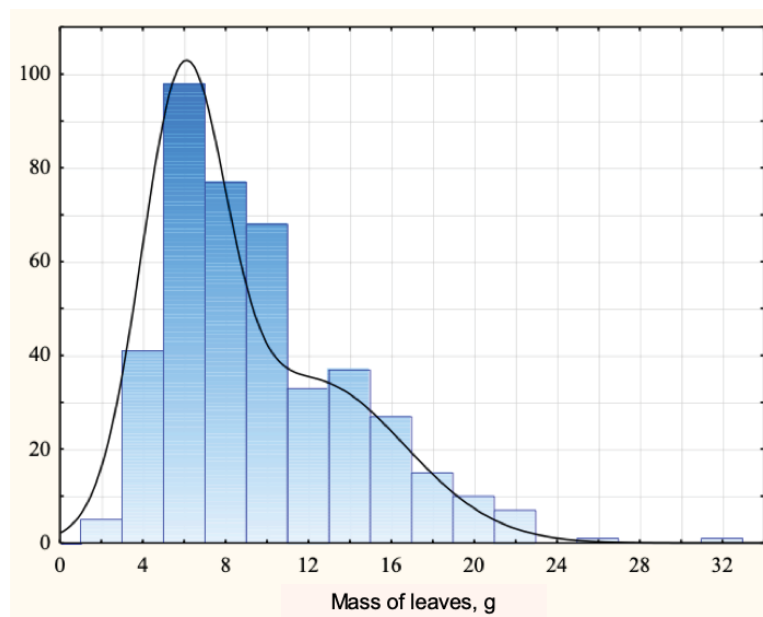


Fig. 5 - Variation in the weight of the stems of *Echinacea pale* depending on the year of harvest and type of pre-sowing seed treatment

The nature of the influence of pre-sowing seed treatment on the variation of stem weight has an annual specificity. Thus, the most pronounced effect of treatments was established for 2013, when the total level of stem mass was the highest. The decrease in the total level of stem mass is associated with a decrease in the effect of treatments on this indicator. The smoothed nature of the effect is set for 2016 and aligned for 2015.

The weight of the leaves of *Echinacea pale* during harvest varies from 0.60 to 30.74 g with an average value of  $8.79 \pm 0.22$  g. Analysis of the histogram of the distribution shows that the leaves form a mixture of several normal distributions (Fig. 6).



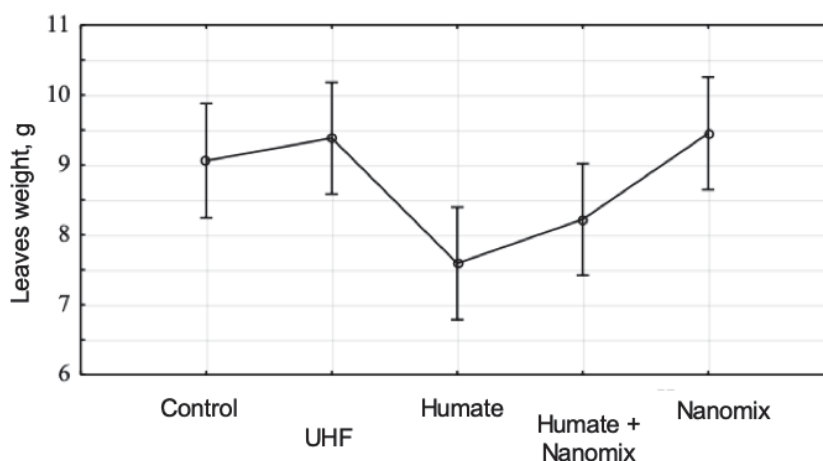
**Fig. 6 - Histogram of leaf mass distribution.**  
The line indicates a hypothetical mixture of two Gaussian distributions

The mixture of three distributions well describes the experimental histogram (Kolmogorov-Smirnov statistics  $d = 0.02$ ,  $p = 0.99$ ). The first component is 34.05% of the sample and is characterized by an average value of  $4.85 \pm 1.45$  g. The second component is 21.59% of the sample and is characterized by an average value of  $7.82 \pm 1.48$  g. The third component is 44.36% of the sample and is characterized by an average value of  $12.28 \pm 4.48$  g.

The mass of leaves correlates with the height of the stems ( $r = 0.61$ ,  $p = 0.00$ ), their number ( $r = 0.39$ ,  $p = 0.00$ ), the mass of the stems ( $r = 0.67$ ,  $p = 0.00$ ) and the number of leaves ( $r = 0.75$ ,  $p = 0.00$ ).

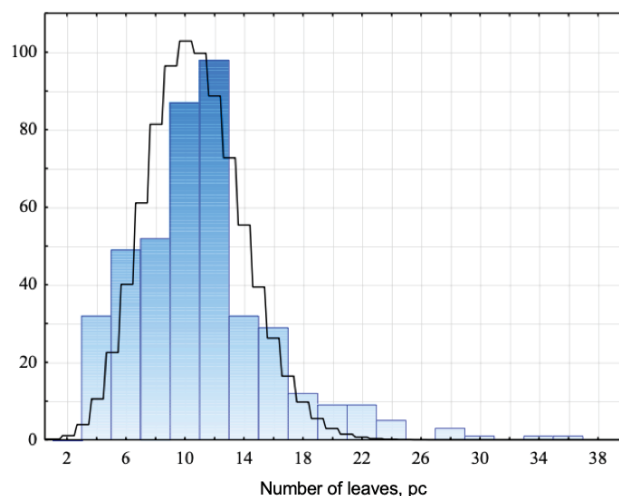
The general linear model of the year and type of seed treatment can explain the 34% variation in leaf weight at harvest ( $R^2 = 0.34$ ;  $F = 16.40$ ;  $p = 0.00$ ).

The year and type of treatment are statistically significant predictors, and their relationship is not a reliable predictor. This indicates that the nature of the effect of pre-sowing seed treatment on leaf mass is invariant and repeated every year. In the control, the leaf mass is  $9.06 \pm 0.42$  g. The greatest stimulating effect was found for Nanomix ( $9.45 \pm 0.41$  g) and UHF irradiation ( $9.38 \pm 0.41$  g) (Fig. 7). The use of humate and a mixture of humate and Nanomix leads to a decrease in leaf weight to  $7.59 \pm 0.41$  and  $8.22 \pm 0.41$  g, respectively. The highest level of leaf mass was set for the 2013 harvest, and the lowest - for 2015. It should also be noted that in general, the year-on-year characteristics of the impact of stimulants are not significant. For 2013, a stimulating effect of a mixture of humate and Nanomix was established, while for other years such an effect was not established.



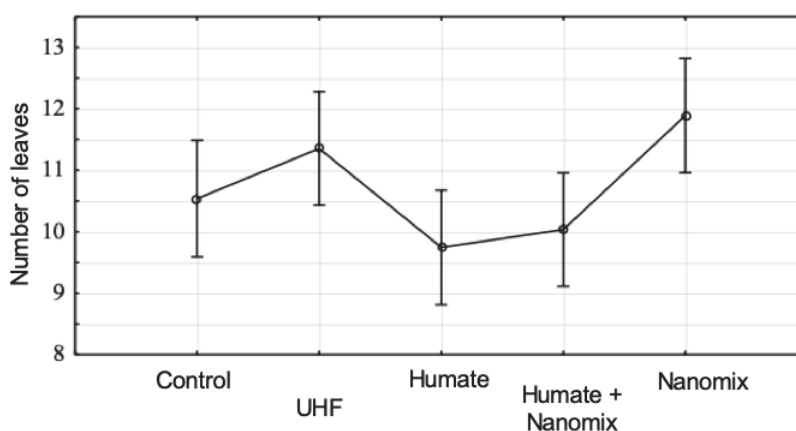
**Fig. 7 - Variation in the weight of leaves of Echinacea pale depending on the year of harvest and type of pre-sowing seed treatment**

The number of leaves on pale echinacea plants during harvest varies from 3 to 36 (median value - 10, arithmetic mean -  $10.66 \pm 0.24$ ). The distribution of the number of leaves differs slightly from the theoretical Poisson distribution due to a shift towards larger values of this indicator (Fig. 8).



**Fig. 8 - Histogram of the distribution of the number of leaves. The line indicates a hypothetical Poisson distribution**

The general linear model of the effect of the year and the type of seed treatment on the number of leaves at the time of harvest can explain 22% of the variation of this trait ( $R^2 = 0.22$ ;  $F = 9.81$ ;  $p = 0.00$ ). It is established that the year and method of seed pretreatment are statistically significant predictors.



**Fig. 9 - Variation in the number of leaves of Echinacea pale depending on the year of harvest and type of pre-sowing seed treatment**

Under control conditions, the number of leaves is  $10.54 \pm 0.48$ . The greatest stimulating effect on this indicator is exerted by seed treatment with Nanomix (Fig. 9). In plants grown after such seed treatment,  $11.88 \pm 0.47$  leaves were found to be present at harvest. Slightly less effective application of UHF radiation -  $11.35 \pm 0.47$  leaves. It should be noted that the differences between these pre-treatment methods are not statistically significant (Mann-Whitney test  $Z = 0.84$ ,  $p = 0.40$ ). The use of a mixture of humate and Nanomix and humate alone did not have a statistically significant effect compared to the control ( $Z = 0.56$ ,  $p = 0.57$  and  $Z = 1.07$ ,  $p = 0.28$ , respectively). Between the years, there are statistically significant differences in the number of leaves on the plants of Echinacea pale during harvest. In 2013, the number of leaves was the largest ( $13.01 \pm 0.35$ ), and in 2015 - the smallest ( $7.72 \pm 0.35$ ). In 2016, this indicator had an intermediate value ( $11.40 \pm 0.39$ ). Trends in the effect of growth stimulants on the number of leaves between years are almost unchanged, which confirms the lack of statistical probability of the predictor Year \* Type of treatment.

## CONCLUSIONS



Pre-sowing seed treatment affects the growth and development of Echinacea pale plants in such a way that this effect is manifested in differences in morphometric characteristics that determine the yield of this crop. Discrete predictors such as the method of pre-treatment, interannual features and their interaction are able to statistically explain 55, 7, 53, 22 and 34% of variations in plant height, number and weight of stems, number and weight of leaves, respectively. The greatest stimulating effect on the height and weight of the stems, the weight of the leaves has a pre-treatment of seeds with nanomix micro-fertilizer or UHF irradiation. The number of stems does not depend on the methods of pre-sowing seed treatment.

The nature of the effect of stimulants on stem height, weight and number of leaves is similar between years, and the effect on stem weight has annual characteristics. The influence of humate or a mixture of humate and nanomix may not manifest itself in the morphometric parameters of plants, or may even suppress them.

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