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ORIGINAL PAPER

Regulating the plant density influences weed infestation, productivity and chemical composition of seeds of true hemp *Cannabis sativa* L.

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Abstract

The article focuses on the cultivation of hemp (*Cannabis sativa* L.) for seed production, specifically examining the effects of different seeding densities and herbicidal control on weed infestation, plant characteristics, and nutrient content in the seeds. Hemp is a versatile plant known for its fiber and oil production, including health-promoting cannabinoids. The study utilizes a Romanian monoecious variety called Secuieni Jubileu, which has low THC content and is suitable for seed cultivation. The experiment compares three treatments: sowing at a density of 20 kg ha^{-1} with herbicide, sowing at a density of 30 kg ha^{-1} with herbicide, and sowing at a density of 30 kg ha^{-1} without herbicide. The results indicate significant differences in weed density among the treatments, with herbicide application and higher seeding density reducing weed populations. Plant height, number of leaves, and seed fraction also varied significantly among the treatments, with higher seeding density (30 kg ha^{-1}) resulting in higher plants, decreased leaf number and with the predominant fraction of 2.5 mm in the seeds. The experimental factor also significantly differentiated the macro- and micronutrient content of hemp seeds. Nutrient content analysis revealed variations in nitrogen, potassium, phosphorus, and magnesium levels in the hemp seeds, depending on the experimental treatments. Overall, the study highlights the importance of seeding density and herbicidal control in hemp cultivation for seed production. The findings suggest that higher seeding density can effectively suppress weed growth, whereas the yield and the high ratio of seed fraction at 2.8 mm is attributed to herbicide control and seed density of 20 kg ha^{-1}. The research contributes to the understanding of optimal cultivation practices for hemp seed production, aiming to support stable, efficient, and profitable agricultural production in the field.

Keywords: hemp, herbicides, sowing density, macroelements, weed control

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INTRODUCTION

Hemp (*Cannabis sativa* L.) belongs to the cannabis family Cannabaceae. This species is derived from wild cannabis that originally grew in Central Asia and south-eastern Europe (Jagmin 1949). There are two subspecies of hemp - Cannabis sativa ssp. sativa and Cannabis sativa ssp. indica. Some sources also provide a third taxon, the wild cannabis (*C. ruderalis*, *C. sativa* var. *spontanea*) – Dzierżanowski (2018). In the pre-Slavic era in Poland, hemp was mainly a fiber plant, and the remaining products (e.g. seeds) could be called the side-products of hemp production (Podbiełkowski 1992). Nowadays, hemp is widely grown not only for fiber, but also to obtain hemp oil, which contains large amounts of health-promoting cannabinoids (Pellati et al. 2018).

Due to the content of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), we divide cannabis into three separate phenotypes: (i) narcotic – when the THC content is above 0.5% and the CBD content is below 0.5%, (ii) indirect – in the case of this phenotype it is difficult to set strict limits, because the proportions may be different (the amount of CBD is dominant, but THC is also present in different concentrations), (iii) fibrous – cannabis varieties with a reduced amount of THC. For the cultivation of industrial hemp to be legal, the THC content should be no more than 0.3% (Commission Regulation (EU) 2022). It should be emphasized that THC, the content of which largely determines the narcotic properties of cannabis, in its natural form is not a psychoactive substance (Horanin and Bryndal 2017, Silska 2017). The original form of THCA (Δ9-tetrahydrocarboxylic acid) turns into psychoactive THC only when the cannabis is exposed to high temperatures, such as when boiled or heated (smoked). Moreover, THC can reduce muscle tension, and has anti-inflammatory and analgesic properties (Truta et al. 2009).

Hemp has phytoremediation properties, thanks to which it is perfect for a polluted environment (Cierpucha et al. 2013, Mańkowski et al. 2014). Currently, soil conditions can be improved through the use of proper practices, therefore this plant will grow wherever we take care of the appropriate content of the humus layer, nitrogen and lime content (Grabowska et al. 2007). Deep, well-drained soils with a water level above 800 mm, neutral and slightly alkaline pH, well insulated and free from water stagnation are optimal condition for its cropping (Ronkiewicz 2018). Hemp has an extensive root system, which can reach up to 3 meters into the ground, which makes it possible to minimize the risk of drought stress, as the plant can freely take up water accumulated in the deeper layers of the soil (Zadrożniak et al. 2017). As a thermophilic crop, during the vegetation period – which lasts on average 135 days – hemp can absorb heat reaching the sum of the effective temperatures of 3,000 degrees Celsius. However, to ensure water demand of cannabis during the growing season, rainfall of 250-300 mm is needed.

The optimal sowing depth is 3-4 cm (Amaducci et al. 2008) and to adjust the sowing density to the expected yield of seed, use of 10 to 15 kg of seeds per hectare should be applied. This value is definitely lower than in the case of hemp cultivation for fiber, where the optimal sowing density is considered to be 60 to 70 kg ha^{-1} (Grabowska et al. 2007).

Weeds in hemp cultivation fields are mainly controlled in two ways: mechanically and chemically. Mechanical weeding requires more work, but is considered an effective method of fighting weed infestation (Robak et al. 2012). The authors proved that mechanical weeding is becoming more and more important because it is considered to be an environmentally safe method, and modern machines mean that at the same time this method allows for a significant reduction of weed populations.

In the case of hemp, an effective and approved weed control agent is Boxer 800 EC. The recommended dose of this agent is from 3 to 4 L ha^{-1} of cultivation. The herbicide is applied approximately three days post sowing in the soil application. The active ingredient of the herbicide is prosulfocarb. Boxer 800 EC is effective in controlling not only dicotyledonous weeds, but it also reduces the population of monocotyledonous weeds (Wójtowicz et al. 2018). Prosulfocarb ($C_{14}H_{21}NOS$) is an active substance from the group of thiocarbamates absorbed in two ways by plants, either through the seed coat or directly through the root system, along with the nutrients. The substance is absorbed by sprouting weeds (Tanski, Idziak 2010).

Prosulfocarb works by blocking the formation of fatty acids so that weeds die quickly. In field studies, it has been shown that the half-life of the agent in soil ranges from 6 to 13 days. Ecotoxicological studies prove that this agent – depending on its concentration – shows low or moderate toxicity to animals and humans (Lewis et al. 2016).

Due to the growing demand for organic products and natural medicinal substances (including CBD cannabidiol oil), the cultivation of hemp is again becoming increasingly popular, although the hemp fields still account for only a fraction of a percent of all agricultural land. Chemical weed control methods are effective, but it cannot be denied that they are both costly and can pose a threat to the environment. An alternative to herbicides could be to apply a higher seeding density, which can give cannabis an advantage over expansive weeds. The search for the most effective methods in the cultivation of hemp that ensure stable, efficient and profitable production of its seeds is one of the challenges of modern field production of agricultural crops. Hemp seeds contain a list of physiologically valuable components, thanks to which they are a priority raw material in the production of functional foods. The unique biochemical composition of hemp seeds opens up opportunities for the extraction and use of individual nutrients in the composition of new generation food products (Miščenko, Lajko 2018) laboratory (thin-layer chromatography. In a recent study conducted in Nigeria, a rough analysis of the mineral, phytochemical and functional composition of hemp was investigated. The average values of various parameters for the approximate composition were as follows: moisture content (5.60+0.05), ash content (6.50+0.01), fiber content (18.85+0.20), fat content (30.43+0.03), crude protein (25.45+0.02) and finally carbohydrate (13.12+0.01) for raw samples respectively. Minerals (mg kg^{-1}) include: potassium (8.75+0,01), calcium (18.60+0.35), sodium (7.52+0.02), magnesium (14.10+0.03), zinc (2.10+0,02), lead (0.05+0.01). The macronutrients and micronutrients are required for the optimal functioning of physiological and biochemical process in the body (Osanyinlusi, Beauty 2023).

The research hypothesis was that hemp cultivation could be regulated by higher sowing densities with or without herbicide use. The aim of the study was to check the impact of canopy density from 20 to 30 kg of seeds per ha on the weed infestation effect, seed productivity and the content of macro- and microelements in hemp seeds.

MATERIALS AND METHODS

Characteristic of the hemp variety

Hemp of Secuieni Jubileu, a Romanian monoecious variety, was selected for cultivation. This variety is recommended for the cultivation of hemp for seeds. The plant reaches an average height of 1.5 to 2 m and blooms in late July and early August. Thanks to the THC content below 0.2%, it was entered into the European catalog of official varieties of agricultural plants. The CBD content is about 0.5%. The most important features of the Secuieni Jubileu variety declared by the producer are as follows: vegetation growth 100 days, oil content 28-32%, fiber content 30-32%, biomass 60-80 dt ha^{-1}, seed yield 8-20 dt ha^{-1}, TSW 16-18 g.

Experiment

A field experiment in 2021-2022 was located on medium clayey soils, slightly acidic, pH approx. 6.5. Before sowing, content of assimilable forms of minerals (mg 100 g^{-1} of soil) were: phosphorus (P) – 34.0 mg, potassium (K) – 18.7 mg, magnesium (Mg) – 5.8 mg.

Three experimental treatments were studied:

– sowing at a density of 20 kg ha^{-1} with the use of herbicide (+ H),

– sowing at a density of 30 kg ha^{-1} with the use of herbicide (+ H),

– sowing at a density of 30 kg ha^{-1} without herbicide.

A traditional seeder with a row spacing of 12 cm and a sowing depth of 3 cm was used for sowing. The soil herbicide was applied 3 days after sowing. The herbicide Boxer 800 EC (3 L ha^{-1}), whose active substance is prosulfocarb, was used for the weeding treatment. This herbicide is a selective agent, adapted to removing monocotyledonous and dicotyledonous weeds. According to the application label, Boxer 800 EC is the only herbicide dedicated to weed control in hemp cultivation. Hemp was sown in the first ten days of May, harvested in the third ten days of September 2021. The preceding crop was hemp cultivated for seeds. No mineral or organic fertilization was applied before sowing. In the first half of June, a dose of nitrogen Yarabela Extran was applied to the entire area of the experiment at a dose of 150 kg ha^{-1}. A rotary hoe treatment was also carried out on all plots in order to level the canopy and eliminate the newly emerging weeds. The area of each plot was 28 ares and the experiment was carried out in three replicates. Harvesting was done in one stage with a combine harvester at BBCH 93 phase. Yield was determined after pre-cleaning and drying the seeds on a floor dryer to a moisture content below 10%. The thousand seed weight was calculated manually for each plot after reducing each sample on the splitter to a value of approximately 200 grams. The density of hemp plants was measured in 10 repetitions by multiplying the number of plants in one running meter by 8 rows, thus obtaining the density per m^2.

Chemical analysis of plant material

Samples

Seed samples were wet mineralized in concentrated sulfuric acid, and the content of total nitrogen was determined based on a modified Berthelot reaction. In brief, after dialysis against a buffer solution of pH 5.2, ammonia in the sample is chlorinated to monochloramine, which reacts with salicylate to form 5-aminosalicylate. Following oxidation and oxidative coupling, a green complex is formed. The absorption of the complex is measured at 660 nm (Skalar SANplus flow analyser), and total phosphorus is determined with the method employing ammonium molybdate (Skalar SANplus flow analyser). The content of potassium, calcium and sodium was determined by flame photometry, and that of magnesium was assayed with the Atomic Absorption Spectrometry (AAS) method.

The content of Mn, Fe, Zn and Cu in grain was determined by standard atomic absorption spectrometry (ASA), following mineralization in a mixture of concentrated hydrochloric and nitric acids in a 1:3 ratio. ASA was carried out using a VARIAN AA240FS fast sequential atomic absorption spectrometer.

Statistical analysis

Data on hemp plant density, weed infestation, plant height and number of leaves per hemp plant, as well as the content of macro- and microelements were analyzed for compliance of the distribution with the normal distribution using the Shapiro-Wilk test. In the case of deviations from the normal distribution (numbers of the weeds), a transformation by the square root of the second degree was applied. Normalized data were recalculated by analysis of variance in order to verify the null hypothesis assuming no differences between the experimental objects in the intensity of occurrence of individual features. For this purpose, the one-way ANOVA test, F, was used at the significance level of p=0.05. In a situation where the significance of the influence of the tested experimental factor on the intensity of the feature was proven, a comparison of the object averages was performed using the Tukey's HSD test, for p=0.05.

RESULTS AND DISCUSSION

Significant differences in weed density between the experimental objects (F value 3.47 - 32.49; p<0.05) concerned the following species: *Euphorbia helioscopia*, *Thlaspi arvense*, *Artemisia vulgaris*, and *Geranium pusillum* (Figure 1). It can be seen that at the sowing density of 30 kg ha^{-1} without the use of herbicide, three species of weeds definitely dominated the experimental plot: *Thlaspi arvense*, *Artemisia vulgaris*, and *Geranium pusillum* (Figure 1). On the experimental plot with a sowing density of 20 kg ha^{-1} and with the application of the herbicide, a smaller number of weeds counted in individual repetitions was observed. Field bundles appeared most often with an average frequency of 8.4 weeds per m^2. On a trial plot with a seeding density of 30 kg ha^{-1}, herbicide application resulted in an average of 5.3 weeds per m^2 frequency (Figure 1).

Significant differences in our research were shown in the height of plants between the study groups. Plants from the groups with a sowing density of 30 kg ha^{-1} were higher due to the competition for light and differed significantly in height compared to the group with a sowing dose of 20 kg ha^{-1}. The competitiveness for sunlight is confirmed by a highly statistically significant difference in the number of leaves on the lowest plants sown at a seeding density of 20 kg ha^{-1}. Highly significant differences were also found in seed yield. Each group was significantly different from others. The highest yield was recorded in the sowing group of 20 kg h^{-1}, i.e. in the lowest plants. Considering the infestation of plants with the insect *Ostrinia nubilalis*, the tendency of yield decrease with increasing plant height seems to be justified in this case. The analysis of the seed fraction in Table 1 clearly shows a significant difference in the seed size on the plot where 20 kg ha^{-1} was sown and accompanied by herbicide application. At the same time, the smallest share of fine seeds with a fraction below 2.2 mm was recorded in this group. It is visible that the plots treated with the herbicide were characterized by a higher thousand seed weight and a higher yield.

The content of macronutrients was unequally differentiated in hemp seeds depending on the experimental objects. With regard to nitrogen (N), significantly the highest content was obtained in seeds in a sample taken

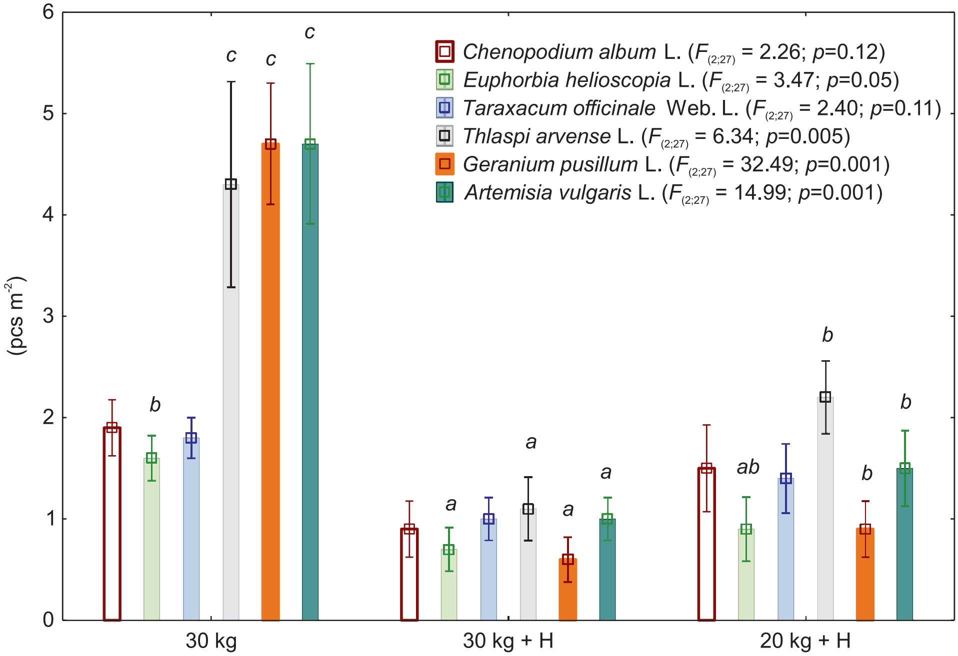

Fig. 1. Occurrence of weeds (pcs. m^{-2}) in hemp cultivation post emergence

from a plot with a sowing density of 30 kg ha^{-1} and with the use of herbicide, i.e. 37.2 g kg^{-1}, and the lowest at a density of 30 kg ha^{-1} without herbicide, i.e. 33.5 g kg^{-1}. Significantly, the highest concentration of potassium (K) was found in samples of seeds from the object with a sowing density of 30 kg ha^{-1} and without herbicide, i.e. 9.5 g kg^{-1}, less at a density of 20 kg ha^{-1} with herbicide, 8.9 g kg^{-1}. In turn, in the case of phosphorus (P), no significant differences were found between the objects, only a tendency to a higher concentration of this element at the sowing density of 30 kg ha^{-1} with the use of herbicide, i.e. 12.4 g kg^{-1}, and lower at the same density without herbicide, i.e. 11.3 g kg^{-1}. Significantly the most magnesium (Mg) was found in the sample taken from the experimental object with a sowing density of 30 kg ha^{-1} (with and without herbicide application), on average 5.73 mg kg^{-1}, the lowest at a density of 20 kg ha^{-1} with herbicide, i.e. 5.04 mg kg^{-1}. Similarly to magnesium, the content of zinc (Zn) was preserved, the highest concentration of which (22.6 mg kg^{-1}) was confirmed in a sample taken from the experimental object with a sowing density of 30 kg ha^{-1}, regardless of the herbicide, and the lowest concentration at a density of 20 kg ha^{-1} after herbicide application (19.8 mg kg^{-1}). The microelements iron (Fe) and manganese (Mn) showed similar differences depending on the sowing and protection against weeds in hemp. Indeed, the highest content of these elements was found in seeds in a sample taken from an experimental object with a sowing density of 20 kg ha^{-1} after herbicide application (Fe – 38.8, Mn – 32.5 mg kg^{-1}),

Table 1

Hemp biological characteristics depending on the seed density and herbicidal control

Characteristic	30 kg + H	20 kg + H	30 kg	*P*
Plant density	44.9	40.8	47.5	0.42
Plant height	163.8*a*	155.7*b*	163.3*a*	0.008
Number of leaves	20.3*b*	24.2*a*	17.3*c*	< 0.001
TSW	16.9	16.2	15.4	0.12
Seed fraction				
2.8 mm	63.5*b*	78.7*a*	60.7*b*	0.05
2.5 mm	23.7*a*	14.4*b*	26.2*a*	0.02
2.2 mm	7.4	4.4	8.1	0.12
Seed yield	196.0*b*	267.3*a*	64.0*c*	0.001

and the lowest at a density of 30 kg ha^{-1} without herbicide (Fe – 32.3, Mn – 28.8 mg kg^{-1}). Contrary to iron and manganese, the highest concentration of copper (Cu) was detected in a sample taken from the experimental object with a seeding density of 30 kg ha^{-1} without herbicide (4.62 mg kg^{-1}), and the lowest at a density of 20 kg ha^{-1} with herbicide (4.05 mg kg^{-1}). In the case of calcium (Ca) and sodium (Na), no statistical differences were found in their concentration in hemp seeds, only a tendency to a higher content in a sample taken from an experimental object with a sowing density of 30 kg ha^{-1} without herbicide (Figure 2).

Adjustment of the sowing density is very important, and this problem is described by Werf et al. (1995), who studied the effect of density on the development of hemp and the quality of the yield obtained. Hemp was grown at 10, 30, 90 and 270 plants m^{-2}. With 270 plants m^{-2}, the phenomenon of "self-thinning" has been observed in several studies. When using dense sowing, hemp plants competed with each other and some plants died during the growing season. In our research, after the ANOVA analysis, a difference in the height of the plants and the tendency to tillering was noticed. When the sowing density was increased by 10 kg ha^{-1}, the plants were higher, which could have resulted from the competition for sunlight. At a sowing amount of 20 kg ha^{-1}, the hemp plants were shorter but more branchy. The effect of density on the growth of hemp was studied in more detail by Hall et al. (2014). It was not only the sowing density was analyzed, but also other factors influencing the yield of plants were scrutinized, e.g. the content of minerals in the soil, temperature and precipitation during the growing season. Similarly to our research results, a relationship was observed between plant density and their height. The larger the sowing density was, the stronger the plants competed for light and the taller they were. The fact that hemp seed yield is also determined by sowing density is emphasized by Burczyk et al. (2009). When growing hemp for seeds or for food, the researchers observed the highest yields at sowing 30 kg ha^{-1}.

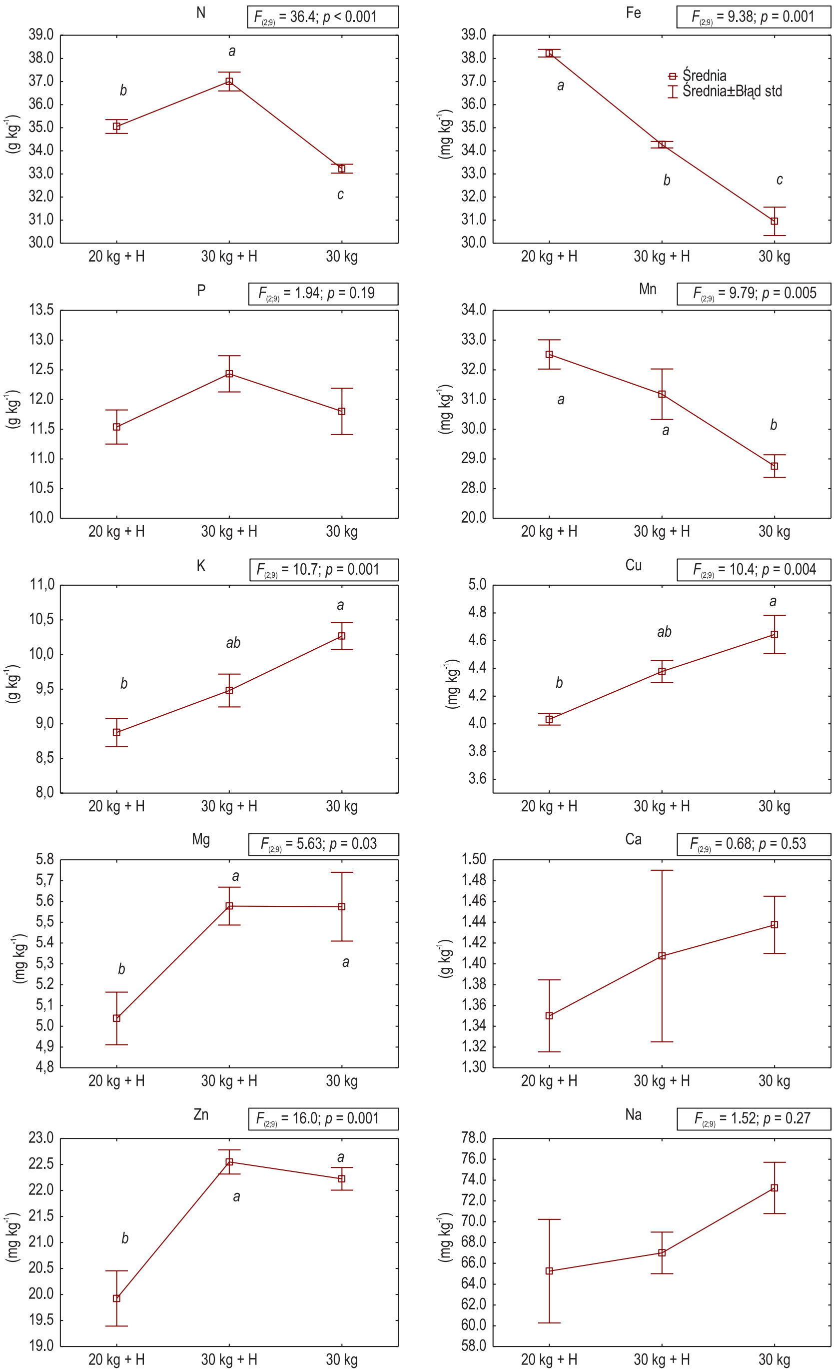

Fig. 2. The content of macro- and micronutrients in hemp seeds depending on the density and herbicidal control

Further increase in the sowing density resulted in a decrease in biomass and seed yield. The results of our research confirmed the observations of biomass capacity, but due to the infection of *Ostrinia nubilalis*, the tallest stems were infected and broke down together with the seed panicle before harvest or during threshing, which resulted in an opposite correlation of seed yield than in Burczyk's research. Turkish research (Yazici 2022) showed that high planting density (200 and 250 plants m^{-2}) had a negative impact on several traits, i.e. plant height, technical stem length, stem diameter, fresh biomass yield, stem dry weight, fiber yield, seed yield and amount of oil. The increase in plant density at a certain rate increased the height of plants and the technical length of the stem. Nevertheless, plant height decreased with the increasing plant density. Due to the height of the plants and the technical length of the stem, the most appropriate planting density was 150 plants m^{-2}. A higher seed yield was recorded for planting 100 and 150 plants m^{-2} than for planting more than 200 and 250 plants m^{-2}. Data in Figure 1 indicate that the number of 3 weed species decreased significantly after increasing the sowing density by 10 kg ha^{-1}, while the number of the remaining 3 weeds did not change significantly. Weeds appeared on all experimental objects. Application of herbicide in the amount of 3 L ha^{-1} significantly reduced weed infestation. There were definitely more weeds on the object where the herbicide was not used. It was necessary to use weed control with a power harrow. In the study of Hall (2014), a lower level of weed infestation was noted on plantations with a larger density. This difference results from the adopted density levels, as in the aforementioned studies the plant density was assumed to be from 100 to 400 plants m^{-2}. The dominant weed (as in our research) was *Chenopodium album* L. The problem of hemp weeding differs not only because of the adopted agricultural technology, but also the location of the crops. Chemical weed control is most effective, but access to plant protection products is limited in many countries. There are still few herbicides approved for use in hemp cultivation on the market (Anderson 2018). In our research, Boxer herbicide was used, which was the only one officially registered as an agent approved for the protection of hemp plantations. In his research, Anderson emphasizes that the major impact on reducing the weed population is the preparation of the soil before sowing (thorough weeding) and control of the level of weed infestation during the growing season, using additional mechanical methods if necessary. In our research, as an additional element limiting the population of weeds, a rotary harrow was used, which effectively eliminated weeds. Recent studies (Ortmeier-Clarke et al. 2022) indicate that hemp is very sensitive to most seed and emergent herbicides tested at a single label dose. In their research on the active substances used so far in the cultivation of soybean and corn, they tried to identify those that would potentially be suitable for the protection of hemp in the future. Of the PRE herbicides tested, clopyralid and saflufenacil are potential candidates for cannabis registration and should be further evaluated in the field. Of the tested herbicides, POST clethodim and clopyralid are candidates for potential registration, which warrants further evaluation in field conditions. Anderson (2018) also mentioned the use of crop rotation as a way to combat weeds and improve soil quality. In hemp cultivation, he mentioned winter wheat and soybean as particularly recommended plants for crop rotation. In the cultivation of winter wheat, it is easier to eliminate dicotyledonous weeds, and the cultivation of soybeans additionally helps to enrich the soil with nitrogen. In our research, it was necessary to apply additional nitrogen fertilization to improve the vitality of plants after emergence.

In this research, iron (Fe) and manganese (Mn) accumulated in the largest amounts after sowing 20 kg ha^{-1} with herbicide, while potassium and copper – after sowing 30 kg ha^{-1} without herbicide. This relationship is inversely proportional and mainly depends on the sowing density factor. According to the total elemental composition (Trukhachev et al. 2022), the results confirmed that hemp seeds are rich in macro-and microelements (in % by weight) including carbon (48 - 49%), oxygen (45 - 48%), silicon (0.1 - 0.4%), calcium (0.3 - 4.7%), potassium (0.1 - 1.0%), magnesium (0.1 - 0.7%), sodium (0.1 - 0.2%), sulfur (0.1 - 0.3%), phosphorus (0.1 - 0.2%), chlorine less than 0.1%.

Research results (Struk et al. 2022) indicate 20 inorganic elements in *Cannabis sativa* L. seeds, oil and pomace. The content of macro-and microelements in the raw materials of *Cannabis sativa* L. corresponds to the following order: Ca>Mg>Si>Fe>Al>Mn>Zn>Sr>B>Cu>Ba>Cr.

A similar order was also observed in our research. The exception in this study is the content of Mg. Struk et al. (2022) state that the largest amount of macro-and micronutrients is accumulated in hemp pomace, and the least – in hemp oil. Thus, hemp oil contains macro-and microelements and can be used as their source in human nutrition. Hemp is one of the plants that easily extract metals such as cadmium. Although above a certain K value in the soil, hemp will more easily transfer K cations instead of Ca. An increase in the K content in the seeds determines an increase in the Mg content so that the K:Mg ratio remains within certain limits. Fertilization does not significantly change the content of metals such as Fe, Mn, Zn and Cd.

CONCLUSIONS

1. Seeding density significantly influenced weed infestation, with higher densities and herbicide application reducing weed populations effectively.

2. Plant characteristics, such as height and number of leaves, varied significantly among treatments, with higher seeding density resulting in taller plants and reduced leaf numbers.

3. The predominant seed fraction at 2.5 mm was observed in higher seeding density with herbicide treatment, indicating potential benefits for seed quality.

4. Nutrient content in hemp seeds showed variations in nitrogen, potas-

sium, phosphorus, and magnesium levels based on the experimental treatments.

5. The combination of herbicidal control and seeding density of 20 kg ha^{-1} led to higher seed yield, suggesting practical benefits for hemp seed production.

6. The research highlights the importance of optimizing seeding density and using appropriate herbicidal control to achieve successful hemp seed cultivation.

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