The Changes of Willow Biomass Characteristics During the Composting Process and Their Phytotoxicity Effect on Sinapis Alba L.

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**The changes of willow biomass characteristics during the composting process and their
phytotoxicity effect on *Sinapis alba* L.**

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Abstract

This study evaluated the use of willow chips for compost production and its effect on *Sinapis alba* L. germination index and seedling growth. A compost experiment with willow biomass was conducted to study its effect on selected compost indexes (particle structure in %, bulk density (kg m^{-3}), and total nitrogen content). The quality assessment of the willow composts was determined after six months of composting process based on the N content and morphological characteristics of tested plant in vegetative chamber. *Sinapis alba* L. was germinated on a water extract made from willow compost using the following additives to willow biomasses: W0 - without additives, WN - with the addition of nitrogen, WF - with the addition of mycelium, WNF - with the addition of nitrogen and mycelium. During the composting process, samples were taken after each mixing of the bio-mass pile to assess their maturity through the use of a bioassay. Willow biomass did not have a negative effect on biological evaluation parameters, and in some indicators, such as the length of embryonic roots in the VI period of the measurements, it was stimulating (61-84% longer in W0 and WF than in the control). The addition of nitrogen during the composting process, especially in the initial composting period, had a strong inhibitory effect.

Keywords

peat replacement, composting biomass, willow, phytotoxicity, germination, seedling parameters

1. Introduction

The most important task facing humanity is to reduce its negative impact on the environment and, in particular to reduce greenhouse gas (GHG) emissions. One of the ways to achieve this goal is to reduce the exploitation of stored carbon resources in the Earth's crust in opposite increase its sequestration in the top layer of the globe [1].

Humans make widespread use of peat, where the bound carbon stocks (in terms of quantity and on a global scale) exceed those bound by tropical forests (500-700 billion tons of C compared 360 billion tons of C, respectively [2]. Globally, the area of peatlands and other mudlands with a high carbon

accumulation is only 3% of the land [3]. Horticulture, as well as agriculture and forestry, makes extensive use of peatlands for various types of production. It is estimated that 10% of the GHG emitted into the atmosphere comes from exploited peatlands [4]. Joosten and Clarke [5] report that peat extraction for horticultural substrates is carried out on about 2000 km² of peatland, half of which is in Germany and Canada. Peatland drainage leads to degradation and, in conjunction with climate warming, rising temperatures, and drier weather patterns, can lead to excessive mineralization and destabilization of peat C stores and, in extreme cases, fires and incineration [2, 6].

Therefore, research is being carried out related to the search for other organic and mineral materials that could be used as a replacement or additive to horticultural substrates that limit the use of peat [7, 8, 9]. Among the potential alternative sources of raw materials replacing peat may be lignocellulosic biomass obtained from Short Rotation Coppice (SRC), e.g. from *Salix viminalis* L. Shredded wood biomass undergoes the biotransformation and transformation process of the lignin-cellulosic complex into humic and fulvic acids [10]. It is well known that compost constitutes a source of macronutrients, being one of the best methods of utilization of various biomasses, including the difficult to utilize, to reuse it for agricultural, reclamation, or horticultural purposes [11]. The application of compost allows carbon to be sequestered in the form of stable organic matter, increases, and keep soil organic carbon at stable levels, therefore, it is one of the key objectives in the priorities of the European Union's Thematic Strategy for Soil Protection [12]. It is also known to improve the physical properties of excessively hydrated biomass by adding wood waste or sawdust to the composting process [13] or shredded wood waste or wood can be fully composted [10, 14].

Willow is a species of origin in East Asia and has spread to almost the entire world, not only in the Northern Hemisphere (from Japan to North America), but also in Southern Australia and New Zealand [15]. The high adaptability to various habitat conditions as well as the wide range of use favour its spread. Due to the high energy value of biomass (15.2–20.1 GJ/t DW dry weight), it is currently mainly used for energy purposes in some regions [16].

The use of compost in horticulture is limited due to its long-lasting effect and reduced nutrient delivery to crops. One of the most important factors that affect the quality of the compost quality is the proper selection of components for the composting process. Additional substances added to compost should have an appropriate carbon to nitrogen (C:N) ratio [17]. The appropriate selection of components and the ensuring of optimal conditions during the composting process create the possibility of the compost use as valuable product. The prerequisite for good quality compost is the selection of the well-chosen components, not only chemically but also from a physical point of view. The components should ensure the right structure of the biomass that would be composted. In the literature, we can find many experiments with different additions to compost aiming to reduce its phytotoxicity and gain stability gaining [18, 19]. According to Jiang et al. [19], delayed nitrogen addition to compost made from animal manure with acidic substrates can lead to a lack of N loss and improve its quality. Maylavarapu and Zinati [20] however stated that analyzing the critical aspects of the compost additives adding is essential. The addition of different additives to compost can cause contamination and enhance nutrients dependent on additives. The addition of N to compost can limit the effect of N fertilization due to the low rates of mineralization and microbial immobilization [21].

Therefore, in present research N and N plus fungi have been proposed as additives to willow biomass with wide C:N ratio to examine its effect on N content and physical parameters. The studies mentioned above comprehensively capture the effect of compost additives on the phytotoxicity of *Sinapis alba* L. in a germination test.

The average amounts of components of willow wood are (%): extractives - 2.0, cellulose – 54.0 and lignin – 26.3 [22, 23]. Willow biomass is also characterized by different components, including flavonoids, tannins, and salicins that belong to phenolic glycosides [24, 25]. Willow is the main natural source of salicylic acid and is characterised by salicylic glycosides, which are biologically active [26]. Salicylates are mainly found in high concentrations in the bark and leaves of plants, excluding the roots, and are extracted mainly in liquid-liquid systems with limited miscibility.

We hypothesized that willow composting would change all the examined features of this material, as well as morphological traits and germination index [27]. This article presents the effect of different methods of composting willow chips on the change of selected parameters of compost during its production, as well as the quality of composted biomass assessed by the germination index with the use of the indicator plant *Sinapis alba* L. The impact of the water extracts made from the composting biomass of *Salix viminalis* L. on different biotransformation processes on the plant growth of the tested plant under controlled conditions was determined.

2. Materials and Methods

2.1 Sampling sites and experiment design

The willow biomass (*S. viminalis* L.) was harvested from SRC in Pawłowice, Wrocław (51°11'N, 17°08'E). The plantation was established in 2003 and regularly harvested every three years for energy purposes. Previously harvested in winter 2015. For composting process harvesting took place in 2019 using a special chopper. The willow chips were immediately transported to Research and Didactic station Psary where the composting process started under semi natural conditions by tipping the chips onto the ground with limiting treatments during the composting process to the necessary minimum. The biomass of the willow plant was cut into uniform organic material with the dominant fraction in terms of <1.9 cm (46.3%), following fraction >1.9 cm (42.0%) and below 0.8 cm (11.7%). The cut stems were composted for a period of six months in four biomass piles with different additives. The different additives were added once one month after the start of composting. These additives comprised: nitrogen in a form of NH_4NO_3 (WN), nitrogen in a form of NH_4NO_3 + fungi (WNF) and Fungi (WF). A control file has also been created without any additives (WO) (Table 1). During composting processes, the temperature and biomass was monitored and complete composting was established on temperature to surrounding outdoor temperature. In this experiment, the following properties of willow compost was performed : total nitrogen (%), bulk density (kg m^{-3}) and percentage of fraction particles in the compost (%).

The composting process was modified through chemical and biological additives. The nitrogen addition (chemical) was used to change the wide C:N ratio and the biological addition (fungal spores) was used to accelerate the decomposition of lignin-cellulosic compounds of the biomass. The C:N ratio in willow biomass is broad, ranging from 49 - 51:1 [28] to 118:1 [29]. During the period from March to the end of July, compost production occurred according to the processes listed below and was cyclically subjected to mixing as indicated by the dates below. Additionally, samples were taken two months after the end of the composting process (September):

- Shredded willow chips (W0),
- Shredded willow chips plus nitrogen in the form of ammonium nitrate (34% N), added to change the C:N ratio (WN). For 1 liter of chips to be composted, 14 g of nitrogen was applied,
- Shredded willow chips with the addition of the wood decay fungus, *Peniophora gigantea* (WF). For 1 liter of chips intended for composting, 2.2 ml of 6% water solution of the fungus was used,
- Shredded willow chips with the addition of wood decay fungi and nitrogen (WNF). 14 g of nitrogen and 2.2 ml of 6% water solution of mycelium were added to 1 liter of chips intended for composting.

For technical reasons, the biomass structure and bulk density of the compost were not determined on date VI. Every month, the piles were mixed and immediately after this process (April, May, June, July) the samples were taken to determine percentages of individual biomass fractions, specific compost weight, bulk density, and total N content. Additionally, a sample for only the N content and the biological test was collected in September (after finishing the composting process).

The following samples were taken into account from different dates to determine the germination capacity of the willow biomass, toxicity potential, and bioassay:

Table 1 List of sampling dates along with the duration of the composting process

Time from composting	Date of sampling	Additive to willow compost	Abbreviation
2 days	23.03.19	-	W0
33 days	10.04.19	-	W0
33 days	10.04.19	Fungi	WF
33 days	10.04.19	Nitrogen in a form of NH_4NO_3	WN
33 days	10.04.19	Nitrogen in a form of NH_4NO_3 plus fungi	WNF
64 days	10.05.19	-	W0
64 days	10.05.19	Fungi	WF
64 days	10.05.19	Nitrogen in a form of NH_4NO_3	WN
64 days	10.05.19	Nitrogen in a form of NH_4NO_3 plus fungi	WNF
95 days	5.06.19	-	W0
95 days	5.06.19	Fungi	WF
95 days	5.06.19	Nitrogen in a form of NH_4NO_3	WN
95 days	5.06.19	Nitrogen in a form of NH_4NO_3 plus fungi	WNF
143 days	17.07.19	-	W0
143 days	17.07.19	Fungi	WF
143 days	17.07.19	Nitrogen in a form of NH_4NO_3	WN
143 days	17.07.19	Nitrogen in a form of NH_4NO_3 plus fungi	WNF
189 days	15.09.19	-	W0
189 days	15.09.19	Fungi	WF
189 days	15.09.19	Nitrogen in a form of NH_4NO_3	WN
189 days	15.09.19	Nitrogen in a form of NH_4NO_3 plus fungi	WNF

Samples of composted willow biomass collected at the above dates were subjected to the following parameters.

Particle size determination was performed by sieve analysis, in which compost samples were sieved through sieves of different sizes (0.8-1.9 cm) until an approximately constant amount of retained particles was obtained. The particle size distribution was assessed using special Pennsylvania sieves (with four different fractions: <0.8 cm, 0.8-1.9 cm >1.9 cm and buttock). Fresh samples were weighed and passed through a series of sieves with different hole diameters: <0.8 cm, 0.8-1.9 cm >1.9 cm. The material remaining on each sieve was then weighed, including the material that passed through the sieve with the smallest diameter. The particle size distribution was then calculated as a percentage of each particle size class in relation to the whole mixed sample.

The bulk density of the willow chips was determined using fresh material from piles. The bulk density of the willow was determined using the mass per unit volume technique [30] with a glass beaker, previously weighed. Bulk density determinations were performed "in situ". During the sampling procedure, the beakers were filled to the mark with the material sampled. Three measures were performed depending on the heterogeneity of the material sampled and the repeatability of the results obtained. The bulk density (**BD**) was determined for each sample of the compost variant and expressed in kg m⁻³ according to formula 1:

1)

$$\text{BD} = \frac{\text{Fresh compost mass}}{\text{Beaker volume}}$$

The sample was weighed and the bulk density was calculated. This procedure was performed for three random samples of fresh material to obtain an average value.

2.2 Preparation of willow extracts

Composted willow wooden chips from piles were taken separately, dried and ground in a mill with a diameter of 0.1 mm sieve. From each term prepared in four replicates 5 g of biomass material (dry weight) and soaked in 100 ml of distilled water. The composting biomass of the willow was kept for 24 hours in darkness, then the extract was filtered using Whatman filter paper no. 1 to remove fiber, lees, and other contamination. The sterilized distilled water was used as the control solution.

2.3 Germination experiments

Germination experiments were performed twice in 2020 and 2021 in 10 cm diameter Petri dishes. Each dish was lined with a single sheet of Whatman No. 1 filter paper and moistened with 5 ml of a particular experimental willow aqueous extract. Each Petri dish before starting the experiment was sterilized at 105°C for 4 hours and seeded with 50 seeds of the tested species *Sinapis alba* L. – white mustard. In the testing phase in both series, there were 96 dishes with the same concentration of willow water extract (5%) and 4 dishes with sterilized distilled water (control treatment). Petri dishes with seeds were placed into a vegetation chamber with 90% humidity. Germination experiments were carried out for seven days, maintaining a temperature regime of 22°C for the constant temperature of

the day (12 h) and 19°C at night (12 h). After three days and the seventh day the germination index was performed. Three days after the beginning of the experiment in the vegetation chamber, 3 ml of the extract (and sterilized water in the control variant) were added to the Petri dishes.

The percentage of germination was calculated for each Petri dish separately (Eq. 1). A germinated seedling was found to be if radicles emerged from the seed [31]. The length of the root (cm), the length of the cotyledons (cm), and the mass of the seedling (g) was measured for selected 10 seedlings per Petri dish. The fresh mass of the seedlings was determined for 10 seedlings to evaluate the allelopathic/toxicity potential of the extract. The experimental results comprised the following parameters:

The number of seeds germinated in each treatment; the percentage of germination in each variant (%). The germination seeds (GS%) were determined by equation (2) [32].

2)

$$GS (\%) = \frac{\text{Number of seed germinated}}{\text{Total number of seed plated}} \times 100$$

Biometric parameters: the embryonic root length (cm), hypocotyl length (cm), and seedling weight (g);

Effect of inhibition according to the formula (Eq. 3) for the II date of compost samples collection only:

3)

$$IE = \frac{C - T}{C} \cdot 100$$

C - measurement at the control variant for *Sinapis alba* L.

T – measurement at each treatment

In the conducted research, cultivated crops (willow and white mustard) were used. According to the national regulations, the use of these species for experimental purposes does not require any special permit. Our study complies with relevant institutional, national, and international guidelines and legislation.

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2.4 Statistical analysis

Changes in nitrogen content, bulk density, and particle size distribution of the samples over and after composting were analysed using Fisher test with LSD to assess the effect of composting on different dates for willow chips variants. Differences between variants were determined by analysis of variance at the significance level of 0.05 or 0.01. In the variance analysis, in (four) replications, repeated in each variant of composting. The calculations (average) were performed with Statistica 13.1. The correlation coefficient (R) was determined with Microsoft Excel 2010. The correlation between total nitrogen and morphological traits of *Sinapis alba* L. has been performed by Pearson correlation.

3. Results and Discussion

3.1. Characteristics of Willow Composting Biomass

During the 6-month composting process, the biomass sampled was characterized by varying nitrogen content (Figure 1). Throughout the sampling period, the N content was highest in the WN variant and ranged from 3.43 (at date VI) to 10.21% (one month after piles formation). The N content was similar when the willow was mixed with nitrogen and mycelium WNF (range from 3.43 to 8.48%). Willow biomass without additives or mixed with mycelium had a similar nitrogen content throughout the study period and ranged from 1.08 to 1.35% (W0) and from 1.18 to 1.53% (WF).

*before composting, LSD Least Significant Difference

Figure 1. The effect of composting processes on total N content (%) for different terms of sampling and variants of composting

The composting process is being regulated by basic environmental factors such as temperature, humidity, aeration, pH, and some properties (such as size of particles and nutrients) of the organic material [33]. Especially the carbon to nitrogen ratio (C:N) is important in composting because microorganisms need a good balance of carbon and nitrogen (between 25 and 35) to be active. An indicator both too high and too low is not appropriate [31]. Ensuring an optimal C:N ratio allows one to gain balance between basic components used by the microorganisms during the composting process; however, not always possible to be fulfilled especially when materials that are added to the composted biomass, leads to the formation of substances having a negative impact on enzymatic activity of microorganisms [34]. Natural composting processes can change the carbon: nitrogen (C:N) ratio during storage [35] leading to changes in the amount of particles less than 3.15 mm [36]. Based on the data from the literature, this ratio is very broad in willow and is approximately 50:1 [28]. Wichuk

and McCartney [37] report that the optimum ratio should be in the range of 10:1 to 15:1. Sullivan et al. [38] recommend the use of nitrogen when woody biomass is composted and the ratio is greater than 20:1. In the study by Whittaker et al. [35], a significant ($p < 0.001$, t-test) increase in nitrogen (N) content was observed in the stored willow material (0.45%) compared to the fresh material (mean 0.31%), while carbon (C) content did not change therefore a lower C:N ratio in the stored material.

Various substrates can be added to the waste during the composting process including mineral, organic, biological, or a mixture of the above substrates [39, 40]. Composting willow with the addition of nitrogen and mycelium contributed to a faster reduction in nitrogen content as a result of biomass decomposition and mineralization. In Jain et al. [33] all elements (N, P, K) increased during the composting process. During the initial phase of composting, nitrogen is generally not used by microorganisms at the same rate as carbon due to significant organic decomposition. As a result, the total nitrogen determined by Kjeldhal (TKN) increases as a concentration effect, but in the study of Chan et al. [41] the increase was not significant similarly to our results where a higher N content in the compost was observed in the variants with nitrogen addition. Chen et al. [42] in the study where added struvite salts increased the nitrogen content in the final compost, but C:N ratio decreased during the composting period as well as in our study. Khater [43] revealed that total nitrogen values ranged from 0.95 to 1.68% for the different types of compost. These results are not in agreement with Benito et al. [44], with the total nitrogen values in compost ranging from 0.99 to 2.01%. In our results, the N content was in a wide range from 1.1% to 10% on nitrogen-containing variants.

Modification of the composting process affected changes in the structure of the compost (Figure 2). The percentage of particle fractions in compost without additives in the second and third time periods was at a similar level. In the later period, the percentage of fractions >1.9 cm decreased, while the highest increase was observed in the percentage of fractions 0.8-1.9 cm. Similarly, a change in the structure of the biomass occurred after nitrogen application, and at the second date a decrease in the percentage of the largest fraction was observed, while the increase in the percentage of fractions 0.8-1.9 and <0.8 cm was at a similar level. The most dynamic changes in structure occurred after the

application of fungi (WF) and the percentage of fractions >1.9 cm successively decreased, while the percentage of the other two fractions increased. At the end of the measurement period, the percentage of fractions <0.8 cm was at the level of the initial values. In the WNF variant, the change in biomass structure was similar to that of the WF, but a smaller increase in the percentage of fraction <0.8 cm was observed.

Figure 2. The structure of the compost biomass changes over the composting process with various additives to the compost. Percentage changes in the beginning of the biomass structure process (in different composting dates and variants)

Chipped willow biomass has a particle structure that does not conform to the optimal parameters for a horticultural substrate. Peat, a standard substrate used in horticulture, has a dominant fraction below 1 cm [45]. It was found that the composting process, irrespective of

treatment, influenced the reduction in the percentage of fraction >1.9 cm and in the chipped willow, nearly 60% was the fraction below 0.8 cm. The study by Whittaker et al. [35] also found noticeable changes in particle size during the composting period for woodchips. In woodchips, there was a 10% increase in the smaller fraction, 3.15-16 mm, after composting. In contrast, the number of small particles less than 3.15 mm, did not change significantly. In their research, that was related to a significant decrease in the bulk density. In our study, the addition of mycelium and nitrogen with mycelium affected the dynamics of these changes, which had already occurred in the initial stage of composting.

The physical parameters of the compost offer better availability of water and oxygen, which are essential for the survival of microorganisms during the composting process, including bulk density [33]. Mohee and Mudhoo [46] claimed that physical parameters had a strong effect on changes in the quality of the final composting product. The bulk density is the important element that influences the optimal conditions for the development of microbial, the microbial activity, the degradation of organic matter, as well as the change in various mechanical properties, such as strength and porosity [47]. The bulk density of the compost depended on the changes that occurred during the composting. In the biomass of willow without additives (W0), there was a successive increase in bulk density from 179 (I date) to 297 (V date) kg m^{-3} (Figure 3). Willow biomass with mycelium additions after an initially large increase in bulk density to 274 kg/m^3 , the bulk density stabilized at 240 kg m^{-3} on the V sampling date. After application of nitrogen and nitrogen with mycelium, the effect on bulk density was similar on all sampling dates and on the date V the weight of 1 m^3 of compost was at the level of 285 and 292 kg (WNF and WN, respectively). During the biomass biotransformation process, there was an increase in bulk weight from 34 (WF) to 66% (W0). The bulk density ranged from 420 to 655 kg m^{-3} for different types of composts [43]. The highest value of bulk density (655 kg m^{-3}) was found for cattle manure compost, while the lowest (420 kg m^{-3}) for sugarcane compost. According to Jain et al. [33] the bulk density was increased from 312 to 380 kg m^{-3} and the volume reduction decreased significantly. Other studies conducted on the composting of various organic wastes have also shown a similar pattern for

bulk density [46]. The size of the particle of compost materials also plays an important role in increasing the bulk density [48]. A study by Jain et al [33] on the composting of nitrogen-rich organic waste using biochar as a bulking agent also showed an increasing trend in bulk density during the composting process. It is proved that bulk density of compost characterizes with decreasing tendency while the total organic matter of the compost increases. In our study, this parameter is changeable depending on the date of sampling and addition.

Substrate weight is a very important indicator that determines water capacity, water holding capacity as well as labor input in the production process. Khoshand and Fall [45], report that the addition of sand ensures optimal water conditions, provided that its proportion does not exceed 36%. The bulk density of the substrate after addition of sand was within the range of 402-1004 kg m⁻³ (peat 375.4 kg m⁻³). Zoltai et al. [49] determined the bulk density for peat within the range of 150-200 kg m⁻³. The composting process increased the bulk density compared to the initial material (179 kg m⁻³). The highest increase was found in willow compost in which the process was not modified and at date VI it was 297 kg m⁻³ and was in the estimated range for peat.

Figure 3. Changes in bulk density depending on the treatment of willow composting and the sampling date (kg m^{-3}) (average for different terms and variants of composting)

3.2 Bioassay of willow biomass during the composting process

On the basis of analyses conducted to evaluate the impact of willow compost with different additives, a differential response was found on the growth and development of the test seed of *Sinapis alba* L. (Photo 1). The aqueous extract of willow, prepared from biomass sampled before the start of the experiment, did not show a significant negative effect on the germination and morphological parameters of the white mustard seedlings (Photo 1, Table 2).

Control

II date - W0

II date - WN

Photo 1. Germination of *Sinapis alba* L. seeds after 7 days of experiment established for the control and II date for W0 and WN treatment

However, no emergence or a marked inhibition of emergence was observed in the willow biomass extract given to the composting process in the first period of the process. In date II, no germination of mustard seeds was observed in the WN variant extract and in the WNF variant the germination capacity was significantly lower. Similarly, significantly lower germination capacity was found for WNF in period IV. At the last sampling date (VI), regardless of the composting method, the germination capacity was statistically at the same level as that obtained under control conditions. The length of the embryonic roots, despite the large differences, was statistically different. This was probably due to the large variation in rootlet length within the sample. The dependence of stimulation

of embryonic root growth was found on the second sampling date in extracts from W0 and WF composted biomass. In the last sampling period (VI), they were respectively 84 and 61% longer than in the control. This relationship was not found on all sampling dates in the WN and WNF variants.

Inhibition of hypocotyl growth was found when extracts were prepared from biomass from WN and WNF. When nitrogen was added to the composted biomass, the toxic effect disappeared in period V, while the addition of nitrogen and mycelium to the composted willow inhibited the growth of hypocotyl until period VI. Composting willow without additives (W0) and with the addition of mycelium had no negative effect on hypocotyl growth and was 14-25% longer than seeds germinated in water.

Composting of willow with nitrogen addition (WN) as well as with nitrogen and mycelium addition (WNF) in the initial period had a negative effect on seedling weight. From date V onward, the seedling weight in the WN and WNF variants was statistically at the same level as in the control. An inhibitory effect of extracts on seedling weight was found in the earlier sampling periods. Willow compost and willow compost with added fungi had a positive effect on seedling weight and a weight increase of 9-40% was observed compared to the control variant. Willow bark extracts, contain many phytohormones, such as salicylic acid, which show a stimulating effect on root system growth, mitigation of environmental stresses, and flowering [50, 51]. In our study, we found a stimulating effect of willow compost extracts and compared to seedlings obtained under control conditions in W0 and WF extracts, mustard seedlings at all sampling dates had longer embryonic roots and hypocotyl. Such a tendency was not observed when extracts were prepared from WN and WNF compost.

Table 2. Germination capacity and seedlings parameters depend on composting treatment and sampling date (average for different terms of sampling and variants of composting)

Compost treatment	Date	Germination index (%)	Root length (cm)	Hypocotyl length (cm)	Seedling mass (g)
Control (water)		96.8	4.4	2.8	0.52
I term before composting		93.0	2.4	2.8	0.47
W0	II	95.1	4.8	3.5	0.57
	III	94.4	6.6	3.4	0.71
	IV	97.1	5.1	3.3	0.68
	V	95.7	6.3	3.2	0.69
	VI	97.6	8.1	3.4	0.73
WN	II	0.0	0.0	0.0	0.00

	III	91.1	2.0	2.1	0.50
	IV	92.5	0.8	0.9	0.28
	V	89.8	2.3	2.5	0.51
	VI	94.1	3.0	2.5	0.55
WF	II	97.6	5.5	3.5	0.65
	III	95.6	6.6	3.3	0.65
	IV	97.3	5.9	3.3	0.65
	V	95.0	5.9	3.5	0.70
	VI	98.2	7.1	3.2	0.73
WNF	II	49.0	0.1	0.2	0.03
	III	93.4	1.5	1.6	0.40
	IV	73.0	0.2	0.7	0.15
	V	90.1	2.2	1.6	0.46
	VI	97.3	3.3	3.0	0.66
LSD ($\alpha=0.05$)		8.3	n.s.	1.2	0.10

Pearson correlation analysis showed a strong interrelation between the germination capacity and parameters of the mustard seedlings and the nitrogen content of the composted biomass (Figure 4). Too high nitrogen content was toxic and inhibited germination and growth of embryonic roots, hypocotyl and negatively affected seedling weight.

Figure 4. Effect on total N content on the germination capacity and seedling parameters of the tested plant *Sinapis alba* L.

The difference in germination capacity of seeds in different media may be caused by different nutrient contents, as confirmed by Atiyeh et al. [52]. The authors showed differences in plant growth between different composts and vermicomposts. Delgado et al. [53] argued that adequate stability and maturity are extremely important characteristics of poultry compost determining its value as fertilizer showing that composting process reduces its phytotoxicity.

Similar study with *Sinapis alba* and water extract from Sunflower was conducted by Bogatek et al. [54]. The seeds of the control mustard were characterized by high germination capacity (95-100%) after 3 days. The sunflower leaves had an inhibitory effect on the germination of mustard seed. The degree of inhibition increased with the concentration of the extract. In our research inhibitory effect was observed while fungi addition to compost.

Sun et al. [55], Chen et al. [42] and Adamczewska-Sowińska [7] report that excessive nitrogen availability can inhibit germination and initial plant growth. In a study by Zhang et al. [56], nitrogen content had a positive effect on the germination of eight plant species. In our study, the high nitrogen content was toxic to white mustard, and the seeds did not germinate, and the lower nitrogen content in the compost had a positive effect on the initial development of the test plant.

For the results obtained in period II, the inhibitory effect was calculated (Figure 5). Composting of willow biomass with nitrogen in the initial period had a toxic effect on the mustard germination process. A similar relationship was found when the composting process was supported by nitrogen and mycelium. The extract of composted willow without additives and with the addition of mycelium had a stimulating effect on the growth of mustard seedlings and compared to the control, the length of the embryonic roots, hypocotyl, and seedling weight were approximately 20% higher than in the control. Such a relationship was found in the WF compost extract.

Figure 5. Inhibition (+) and stimulation (-) effect of extract from willow composting biomass in percentage of seedling and germination parameters collected at the II term compared to the control (mustard seeds germinated in distilled water).

Compost produced from willow, whose biotransformation process was aided by mycelium, had beneficial stimulating effects on seedling growth. Hayat and Ahmad [50] demonstrated the beneficial effect of salicylic acid on many plant growth processes. The stimulating effect of secondary metabolites

found in willow can be inhibited by the high nitrogen content in the medium, especially immediately after application.

4. Conclusions

The production of willow compost must comply both with the process requirements arising from the appropriate C:N ratio and with the quality of the substrate produced. The addition of nitrogen, used due to its positive influence on the biomass biotransformation process, however can be toxic to plants. Therefore, it is necessary to determine the maximum dose that will ensure the correct course of the mineralization process and will not lead to nitrogen depletion of the composted biomass and inhibition of the process or excessive nitrogen accumulation. Composting woody biomass rich in cellulose and lignin's does not have such a dynamic progression, while the addition of nitrogen enhances its course and in the initial period the nitrogen content is too high for the correct development of the seedlings. With subsequent stages of composting, the nitrogen content decreased and the mature compost (date VI) showed no toxic effect on the test plant. The research showed that the addition of mycelium did not have a toxic effect on the test plant, and the production effect and the beneficial effect on the change in the structure, as well as the biological evaluation confirmed the assumptions adopted. The study showed that in terms of quality, the willow compost fulfilled the quality requirements and the plant test showed in some variants a stimulating effect on the initial growth of white mustard.

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7. Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Anna Jama-Rodzeńska, Peliyagodage Chathura Dineth Perera and Józef Sowiński]. The first draft of the manuscript was written by [Józef Sowiński] and all authors commented on draft versions of the manuscript. All authors read and approved the final manuscript

8. Patents

Some of the results presented in this article were used in a patent filed on 28.06.2020 at the Polish Patent Office No. P.435103. A legal procedure is currently underway.

Data Availability

Authors can confirm that all relevant data are included in the given article.

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