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The temperature influence on the energy and germination capacity of seeds and the effect of the substrate on the yield *the withania somnifera* in the conditions of south-eastern Poland

Wpływ temperatury na energię i zdolność kiełkowania nasion oraz wpływ podłoża na plonowanie *Withania somnifera* w warunkach Polski południowo-wschodniej

Abstract: The aim of this study was to determine the effect of air temperature on the energy and germination capacity of *W. somnifera* seeds and to assess the effect of different substrates on the yield of the aboveground and root parts. An additional aim of the study was to develop agrotechnical recommendations for pioneering cultivation of this species in the temperate climate of south-eastern Poland. Germination energy and capacity were evaluated at 10°C, 20°C, and 30°C. Additionally, the impact of three substrate types – soil (A), a 50 : 50 mixture of soil and compost (B), and pure compost (C) – on the yield of shoots and roots was assessed over the years 2021–2023. The results indicate that the germination energy and capacity of *W. somnifera* seeds were highly dependent on air temperature. The highest germination energy was observed at 30°C in all study years, with an average value of 93.56%,

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while the lowest was recorded at 10°C (average: 0.44%). Germination capacity was also highest at 30°C, reaching an average of 95.45%, indicating that this temperature is optimal for maximizing both germination energy and capacity. Substrate type had a significant effect on the yield of both aboveground and root parts. The highest yield of aboveground biomass was obtained on pure compost (C), while the lowest was recorded on the soil-compost mixture (B). Over the three years, substrate C consistently provided the highest root yields, averaging 3.5 t ha^{-1}, followed by substrate B (3.2 t ha^{-1}) and substrate A (3.1 t ha^{-1}). This study demonstrates that *W. somnifera* has high adaptive potential for cultivation in Poland; however, it requires high air temperature during seed germination and appropriate substrate selection. These results allow for the development of agronomic recommendations for the cultivation of *W. somnifera* in south-eastern Poland.

Keywords: *Withania somnifera* L., seeds, temperature, germination capacity, germination energy, herb yield, root yield

INTRODUCTION

The genus *Withania* includes 23 species, two of which – *Withania somnifera* (ashwagandha) and *W. coagulans* (ashutoshbooti) – occur in India. Their dried roots are widely used in traditional medicine, particularly in Ayurveda [Połumackanycz et al. 2020]. *Withania somnifera*, also known as Indian ginseng, belongs to the nightshade family (Solanaceae) and is a xerophytic plant that naturally occurs in dry, hot regions of the Middle East and North Africa [Khabiya et al. 2024]. It is primarily cultivated in subtropical and tropical zones, including India, Africa, and Australia [Obidowska and Sadowska 2004]. In India, ashwagandha is extensively grown as a medicinal plant, particularly in the northwestern region of Madhya Pradesh, on more than 5,000 ha of land. Other major producing states include Rajasthan, Gujarat, Uttar Pradesh, Punjab, Haryana, Andhra Pradesh, and Maharashtra, with a total cultivation area of approximately 10,768 ha [Khabiya et al. 2024]. In Ayurvedic medicine, ashwagandha is used as an adaptogen to help the body cope with stress, and it also exhibits anti-inflammatory, neuroprotective, and antioxidant properties [Afewerki et al. 2021, Mikulska et al. 2023, Mondal and Paul 2023, Dipankar et al. 2025]. The roots are the most valuable plant part [Chauhan et al. 2022], but a bioactive compound in the leaves, withaferin A, has demonstrated anti-cancer potential, increasing the plant's significance in pharmaceuticals. In India, ashwagandha is often cultivated on marginal soils by small-scale farmers in states such as Madhya Pradesh, Andhra Pradesh, Rajasthan, and Karnataka. Its popularity is due to ease of cultivation, high market value of roots, and additional income from leaves and seeds [Mondal and Paul 2023]. The root is the main pharmaceutical raw material used in Ayurvedic and Unani medicine to treat rheumatic diseases, lung infections, stomach ailments, and skin conditions, and it is valued for its anti-inflammatory and aphrodisiac properties [Połumackanycz et al. 2020]. In recent years, interest has grown in cultivating *W. somnifera* in temperate climates, including Poland, due to increasing demand for natural herbal raw materials [Pisulewska et al. 2025]. However, cultivation under these conditions presents several challenges, such as a shorter growing season, lower temperatures, and reduced sunlight. The minimum germination temperature for this species is 18–20°C, while the maximum is 30–32°C; temperatures outside this range may slow germination or inhibit growth [Singh et al. 2015]. In Poland, *W. somnifera* is cultivated as an annual plant, and cultivation success depends on substrate choice, germination conditions, and agronomic practices. Low seed germination capacity in temperate climates is a major limitation [Kaur et al. 2018], which makes achieving high germination energy and capacity crucial to justify seed costs and ensure profitable yields [Kumar et al. 2016]. The aim of this study was to determine the effect of air temperature on the energy and germination capacity of *W. somnifera* seeds and to assess the effect of different substrates on the yield of the aboveground and root parts. An additional aim of the study was to develop agrotechnical recommendations for pioneering cultivation of this species in the temperate climate of south-eastern Poland.

Alternative hypotheses:

H_1: There is a statistically significant effect of different temperature levels on the germination capacity of *W. somnifera* seeds.

H_2: There is a statistically significant effect of different substrate types on the yield of *W. somnifera*.

Null hypotheses:

H_{01}: There is no statistically significant effect of temperature on the germination capacity of *W. somnifera* seeds.

H_{02}: There is no statistically significant effect of substrate type on the yield of *W. somnifera*.

MATERIALS AND METHODS

The field experiment was conducted over three growing seasons (2021–2023) in the field experiment was conducted over three growing seasons (2021–2023) at the Experimental Field of the State Higher Vocational School in Krosno (49°41'N, 21°47'E) under the climatic conditions of south-eastern Poland. The study was arranged in a randomized block design with three replications. To evaluate the effect of substrate on plant growth and yield, three experimental variants were applied: A (control), consisting of garden soil (S); B, a 50 : 50 mixture of garden soil and compost (S + C); and C, pure compost (C). The clean compost was purchased from Krosno Municipal Holding. Each experimental plot had an area of 13.5 m^2 (9 × 1.5 m).

Seedling preparation and production

The seeds were purchased from a certified seed retailer. Class A seeds were soaked in distilled water for 24 h prior to sowing. They were then sown into multi-cell trays filled with a 50 : 50 mixture of soil and compost, with the top layer covered with perlite to maintain optimal moisture. After reaching the BBCH 19 (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) growth stage (approximately 7 cm in height), the seedlings were transplanted into P9 pots containing the same substrate. The plants were subsequently transplanted to the field at the BBCH 29 stage (about 5 weeks after sowing), maintaining a spacing of 30 × 25 cm. Due to the lack of registered plant protection products for *W. somnifera* in Poland, crop maintenance was limited to manual weeding. Harvesting was carried out in two stages: the above ground parts were collected at the beginning of flowering (BBCH 61), while the roots were dug up at the end of October (BBCH 95). The seedling production process and subsequent stages of cultivation are illustrated in figure 1.

Fig. 1. Steps of growing *Withania somnifera* L**.** A – seeds, B – seedling, C – seedling planted in a plantation, D – flowering, E – tsarina plantation, F – fruit formation, G – above-ground part (stem, leaves, fruits) and underground part (root), H – harvesting of roots

Laboratory analysis and soil studies

Between 2021 and 2023, seed germination tests were conducted at the Microbiology Laboratory of the State Academy of Applied Sciences in Krosno. The seeds were disinfected by soaking them for 24 h in 40% ethanol, followed by 8 h in 3% perchloric acid. The prepared seeds were then placed on sterile filter paper in Petri dishes (150 × 25 mm) (100 seeds per dish, in three replicates). Germination was monitored in a Fito 300 incubator (Biogenet, Poland) at a constant relative humidity of 95%, under temperatures of 10°C, 20°C, and 30°C, with a photoperiod of 16 h light/8 h dark. Germinated seeds were counted every 48 h, and germination capacity and germination energy were determined according to ISTA guidelines [ISTA 2004]. Before the start of each growing season (2021–2023), representative soil samples were collected from the topsoil layer (0–30 cm) of each plot in accordance with the Polish Standard [PN-R-04031:1997] and analyzed at the accredited Regional Chemical-Agricultural Station in Rzeszów. Key chemical parameters were assessed, including pH (in 1 M KCl), organic matter content (Tiurin method) [Myślińska 2010], as well as macroelements (P, K, Mg) [Handzel et al. 2017] and microelements (Cu, Mn, Zn, Fe) [Ostrowska et al. 1991].

Physicochemical properties of soil

Table 1 presents the physicochemical properties of three different types of soil substrates analyzed during the years 2021–2023. These substrates include: A) (Z) – soil serving as the control object, B) (Z + K) – a mixture of soil and compost in a 50 : 50 ratio, and C) (K) – pure compost.

Table 1. Chemical composition and soil reaction (2021–2023): macro- and micro-nutrients, humus, calcium carbonate

Micronutrients (mg kg^{-1})				pH (KCl)	Humus (g kg)	CaCO3 (g kg^{-1})	Macronutrients (mg·kg^{-1})			Substrates
Fe	Zn	Mn	Cu				Mg	K_2O	P_2O_5	
1585	14.6	173.9	5.7	5.69	26.8	0.2	241	310	196	A
1798	15.6	184.1	6.1	6.70	29.2	0.4	198	202	123	B
1887	16.4	186.9	4.7	7.10	29.8	0.6	370	380	240	C

Source: Data prepared based on the results obtained from the District Chemical-Agricultural Station in Rzeszów; substrates: A – soil (Z) as a control object, B – soil + compost (Z + K; 50 : 50), C – compost (K)

The analysis shows that substrate C (compost) had the highest content of macronutrients (P_2O_5, K_2O, Mg) and humus compared to the other substrates, reflecting its greater nutritional potential. Substrate C also exhibited the highest pH value (7.10), indicating a slightly alkaline reaction, in contrast to the slightly acidic reaction of substrates A (pH 5.69) and B (pH 6.70). The highest concentrations of iron (Fe = 1887 mg kg^{-1}), manganese (Mn = 186.9 mg kg^{-1}), and copper (Cu = 16.4 mg kg^{-1}) were also observed in the compost substrate. On the other hand, the control substrate A had the lowest content of these elements. Additionally, substrate A showed the lowest levels of both $CaCO_3$ and humus, making it the least nutrient rich. Substrate B (soil + compost) was intermediate in terms of $CaCO_3$ and humus content, while substrate C (compost) stood out with the highest values, indicating the greatest potential for enriching the soil and improving its physicochemical properties (tab. 1). This suggests that the use of compost (substrate C) can significantly enhance plant growth conditions by enriching the soil with essential nutrients and improving its structure. The weather pattern during the growing season of the ashwagandha was variable, as illustrated in table 2.

According to data from the meteorological station in Dukla, the weather conditions during the vegetation period of ashwagandha in the years 2021–2023 were characterized by considerable variability. Three key parameters were analyzed: precipitation, air temperature, and humidity, expressed by the Hydrothermal Coefficient (HTC).

Precipitation

The total precipitation sum for the April–September period was lower than the long-term average. The driest year was 2021, with mean monthly precipitation of 17.9 mm. Exceptionally low precipitation was recorded in June 2022 (6.5 mm) and in July 2023 (11.7 mm), which could have led to water stress in plants. In contrast, the highest precipitation was observed in May 2022 (61.4 mm) and in June 2023 (52.9 mm).

Table 2. Meteorological conditions during the growing season of ashwagandha 2021–2023, according to the Dukla Meteorological Station

Years	Months						Mean (IV–IX)
	IV	V	VI	VII	VIII	IX	
Rainfalls (mm)							
2021	7.1	16.1	14.0	20.9	31.2	41.5	17.9
2022	23.8	61.4	6.5	16.1	13.0	10.2	24.2
2023	23.3	35.7	52.9	11.7	15.3	22.0	27.8
The average sum of 2021–2023	55.9	95.6	100.9	116.5	30.1	53.1	79.8
Air temperature (°C)							
2021	15.5	18.8	20.9	21.3	21.6	13.0	19.6
2022	10.6	13.7	23.0	20.2	20.6	12.4	17.6
2023	10.0	12.4	17.1	20.4	21.3	15.5	16.2
Long-term average (2004–2023)	9.2	13.6	16.4	19.0	19.4	14.0	15.5
Hydrothermal coefficient (HTC)							
2021	0.5	0.9	0.7	1.0	1.4	3.2	0.9
2022	2.2	4.5	0.3	0.8	0.6	0.8	1.7
2023	2.3	2.9	3.1	0.6	0.7	1.4	1.9

The ranges of this index values were classified as follows: $K \leq 0.4$ – extremely dry month; $0.4 < K \leq 0.7$ – very dry; $0.7 < K \leq 1.0$ – dry; $1.0 < K \leq 1.3$ – quite dry; $1.3 < K \leq 1.6$ – optimal [Cherszkowicz 1971]; $1.6 < K \leq 2.0$ – moderately humid; $2.0 < K \leq 2.5$ – humid; $2.5 < K \leq 3.0$ – very humid; $K > 3.0$ – extremely humid [Skowera and Wojnowski 2003]

Air temperature

The average air temperature in 2021–2023 was higher than the long-term mean (15.5°C), which was favorable for the vegetation of the thermophilic ashwagandha. The warmest period was August 2021 (21.6°C). The lowest average temperature was recorded in April 2023 (10.0°C). Despite the overall warming trend, the cool beginning of the 2023 season may have delayed early plant development.

Air humidity

Humidity was assessed using the Hydrothermal Coefficient (HTC), with values categorized according to the classification of Skowera and Wojkowski [2003]. The analysis revealed considerable variability in humidity conditions across months and years. For instance, May 2022 (HTC = 4.5) was a very humid month, which could have positively influenced plant development, whereas June 2022 (HTC = 0.3) and July 2023 (HTC = 0.6) were very dry, posing challenges for cultivation. The overall mean HTC values for the respective years indicated a predominance of dry to moderately humid conditions.

Statistical calculations

The experimental data were analyzed using a two-way analysis of variance (ANOVA) with interaction effects to evaluate the influence of experimental factors (substrate type, meteorological conditions) on response variables (germination capacity, yield, biomass parameters) [Sokal and Rohlf 1995, Quinn and Keough 2002]. For post-hoc comparisons between group means (e.g. different substrates or years), Tukey's Honestly Significant Difference (HSD) test was applied [Tukey 1949, Zar 2010]. This conservative multiple comparison procedure maintains the experiment-wise error rate at $\alpha \leq 0.05$. The results were presented using standard ANOVA tables showing F-statistics, degrees of freedom, and significance levels [Press Underwood 1997], as well as boxplots for visualizing comparisons between groups [McGill 1978, Crawley 2013]. All analyses were performed using the R statistical software [Core and Team 2023] with the Agricola [Mendiburu 2021] and ggplot2 [Wickham 2016] packages for statistical computations and data visualization, respectively.

RESULTS

Seed germination capacity and energy

One of the basic yield-forming factors in plant production is seed material, and seed germination energy and germination capacity are the first steps towards successful cultivation of ashwagandha. The results of seed germination are presented in tables 3 and 4.

Table 3. Germination capacity of ashwagandha seeds at different temperatures (%)

Air temperture	Years			Mean
	2021	2022	2023	
10°C	1.3 ±0.0 [c]	1.7 ±0.0 [c]	1.3 ±0.0 [c]	1.6 [c]
20°C	44.7 ±1.0 [b]	44.7 ±1.2 [b]	44.7 ±1.0 [b]	44.6 [b]
30°C	96.0 ±1.1 [a]	95.7 ±2.2 [a]	96.0 ±1.1 [a]	95.5 [a]
LSD $p \leq 0.05$	7.22			2.4
Mean	47.3[a]	47.3 [a]	46.9[a]	47.2
LSD $p \leq 0.05$	ns*			–

Statistically significant differences in means within groups (lines) are marked with letters,
± – standard deviation,
* not significant at $p \leq 0.05$

The germination capacity of ashwagandha significantly varied depending on the air temperature, which means that the temperature significantly affected the results obtained. The highest germination capacity was recorded at 30°C, reaching an average of 95.45%, which indicates that this is the most optimal temperature for germinating ashwagandha seeds. At 20°C, the germination capacity was on average 44.56%, which is significantly lower than at 30°C, but still significantly higher than at the lowest air temperature. The lowest germination capacity was recorded at 10°C, where the results were almost zero (1.6%). This shows that such a low temperature is not suitable for germinating this plant (tab. 3). Differences between years: the average germination capacity in 2021–2023 was: 47.3% (2021), 47.3% (2022) and 46.9% (2023), respectively. The differences between years were not statistically significant, indicating that the germination capacity of seeds was not dependent on the variability of conditions in individual years in a statistically significant manner (tab. 3). This study highlights the need to ensure appropriate thermal conditions for optimal ashwagandha growth.

No significant differences were observed between years in individual temperatures, emphasizing that temperature is the main factor determining the germination capacity of seeds. Therefore, the obtained results clearly indicate that for ashwagandha, the optimal temperature for seed germination is around 30°C, which is in line with the literature on this plant, which prefers warm, subtropical conditions. A temperature of 20°C, although it allows germination of some seeds, is not high enough to achieve the full germination potential. On the other hand, a temperature of 10°C completely prevents effective seed germination. For commercial cultivation of ashwagandha, it is therefore recommended to use thermal conditions close to 30°C to achieve high germination capacity and ensure the success of the crop. In conclusion, temperature is a key factor influencing the germination capacity of ashwagandha seeds, and the optimal thermal conditions are 30°C. Ashwagandha seed germination varied significantly under different temperature regimes, with the highest values observed at 30°C, indicating optimal conditions for rapid and uniform seedling emergence. Lower temperatures (10°C) resulted in minimum germination energy, highlighting the species' preference for warmer climate during the initial growth phase (tab. 4). The standard deviation for this feature (e.g. 94.7 ±4.1 for a temperature of 30°C) confirmed that the measurements were very precise and repeatable (tab. 4).

Table 4. Germination energy of ashwagandha seeds at different temperatures (%)

Air temperture	Years			Mean
	2021	2022	2023	
10°C	0.3 ±0.0 ^{c}	0.3 ±0.0 ^{c}	0.7 ±0.1 ^{c}	0.4 ^{c}
20°C	44.7 ±1.2 ^{b}	45.7 ±1.9 ^{b}	45.3 ±1.6 ^{b}	45.2 ^{b}
30°C	92.7 ±3.1 ^{a}	94.7 ±4.1 ^{a}	93.3 ±3.6 ^{a}	93.6 ^{a}
LSD $p \leq 0.05$	7.22			2.37
Mean	45.9 ^{a}	46.9 ^{a}	46.4 ^{a}	46.4
LSD $p \leq 0.05$	ns			–

Statistically significant differences in means within groups (lines) are marked with letters, not significant at $p \leq 0.05$

The results of our own research indicate that the germination energy of ashwagandha seeds was significantly dependent on the germination temperature. The highest germination energy was observed at 30°C in all the years of the study, with an average value of 93.6%, which indicates that this temperature provides optimal conditions for seed vigor and early seedling development. Moderate germination energy was recorded at 20°C (average: 45.2%), while the lowest germination energy was observed at 10°C (average: 0.44%), which highlights the adverse effect of low temperatures on seed metabolic activity and germination potential. LSD analysis confirmed significant differences between temperature treatments, without significant changes between the years of the study.

The interaction between temperature and years indicates that the effect of temperature on germination energy of ashwagandha seeds was stable in the years studied (2021–2023). The results show that temperature was crucial for germination energy, and the highest values were consistently recorded at 30°C regardless of the year, which indicates the repeatability and independence of this result from environmental conditions in individual years (tab. 4).

In contrast, at 20°C, germination energy values were moderate and stable between years, suggesting that this temperature provides acceptable, although suboptimal conditions. On the other hand, at 10°C, the interaction was the least pronounced, as germination energy was very low and similar each year. The lack of significant differences between years (LSD = ns) indicates that germination energy was more influenced by temperature than possible differences resulting from annual conditions.

Yield of the aboveground and underground parts of the Withania somnifera

Soil substrates significantly affected the yield of the aboveground part of the *Withania somnifera*. The highest yield of the tested trait was achieved in the substrate with compost, while the lowest in the substrate made of soil and compost (B). Statistical analysis showed that in the first year of the study, the yield of the aboveground part of *Withania somnifera* significantly depended on the substrate used and was the highest in the treatment with compost. The treatment with compost alone increased the herb yield by 12.4%, compared to the control treatment (A). In the treatment where compost was added to the soil in a 50 : 50 ratio, the value of the tested trait was only higher by approx. 2%, compared to the control treatment (tab. 5). The standard deviation for fresh mass of above ground parts of ashwagandha (e.g. 15.1 ±1.1 for compost substrate) confirmed that the measurements were repeatable (tab. 5).

Table 5. Fresh mass yield of above-ground parts of ashwagandha (t ha^{-1})

Substrates	Years			Mean
	2021	2022	2023	
A	9.7 ±0.9 ^{b}	11.0 ±0.9 ^{ab}	12.1 ±1.0 ^{b}	10.9 ^{c}
B	13.6 ±0.8 ^{a}	12.6 ±1.0 ^{a}	14.4 ±1.2 ^{a}	13.5 ^{b}
C	13.8 ±1.0 ^{a}	14.1 ±1.0 ^{a}	15.1 ±1.1 ^{a}	14.4 ^{a}
LSD $p \leq 0.05$	2.49			2.37
Mean	12.4 ^{b}	12.6 ^{b}	13.9 ^{a}	13.0
LSD $p \leq 0.05$	ns*			–

Statistically significant differences in means within groups (lines) are marked with letters, ± – standard deviation, substrates: A – soil (S) as a control object, B – soil + compost (S + C) (50 : 50), C – compost

Year × substrate interaction is a statistical term used in analysis of variance (ANOVA) that refers to a situation in which the effect of one factor (in this case, year) on the outcome of an experiment depends on the level of a second factor (in this case, substrate). The interaction between substrates and years in relation to the fresh mass of the above-ground parts of ashwagandha indicates a varied response of plants to the type of substrate in individual years (fig. 3). Substrate A (control object): Fresh mass was the lowest in all years, confirming that soil alone (without compost addition) does not provide optimal conditions for maximum yield. However, an increasing trend was observed in subsequent years (from 9.7 t ha^{-1} in 2021 to 12.1 t ha^{-1} in 2023), which may suggest improved environmental conditions or the influence of increasing plant adaptation. Substrate B (soil + compost, 50 : 50): The test results were higher than in the control substrate in each year. The average fresh mass increased from 13.6 t ha^{-1} in 2021 to 14.4 t ha^{-1} in 2023, indicating that compost addition had a positive effect on yield and that the effectiveness of this substrate was stable between years. Substrate C (compost): This all-compost substrate provided the highest fresh mass in each year, with a peak yield of 15.1 t ha^{-1} in 2023. These results suggest that compost as a substrate provided optimal growth conditions, likely due to its high nutrient content and improved physical soil properties (fig. 3).

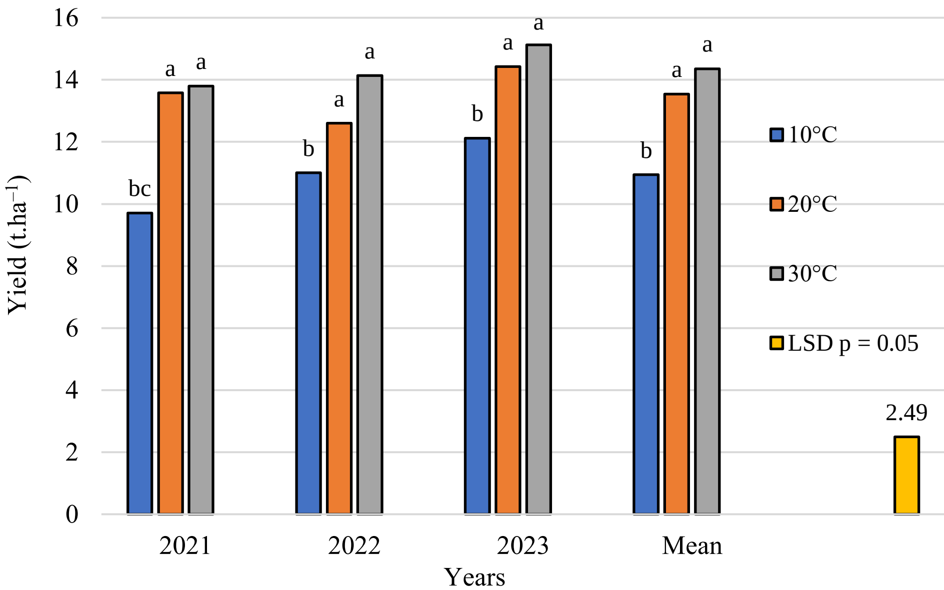

Fig. 3. Interaction of substrates and years of research on the yield of aboveground biomass of ashwagandha

Year and substrate interaction: Although there was an overall improvement in yield between years, regardless of substrate, the difference between substrates was significant. Substrate type had the greatest effect on fresh mass, with years having only a moderate effect (fig. 3). This suggests that the main factor influencing yield is the soil quality, while environmental conditions in individual years played a secondary role.

Table 6 shows the dry matter yield data of the above-ground parts of ashwagandha (t ha^{-1}) over three years (2021–2023) under different substrate conditions (A, B and C). The standard deviation for dry matter of above-ground parts of ashwagandha (e.g. 3.1 ±0.1 to 3.4 ±0.3) for a compost substrate) confirmed that the measurements were repeatable (tab. 6).

Table 6. Yield of dry matter of above-ground parts of ashwagandha (t ha^{-1})

Substrates	Years			Mean
	2021	2022	2023	
A	2.0 ±0.1^{c}	2.0 ±0.2^{b}	2.9 ±0.3^{b}	2.3^{c}
B	2.5 ±0.1^{b}	2.5 ±0.2^{b}	3.1 ±0.2^{a}	2.7^{b}
C	3.1 ±0.1^{a}	3.2 ±0.1^{a}	3.4 ±0.3^{a}	3.3^{a}
LSD $p \leq 0.05$	0.4			2.37
Mean	2.5^{b}	2.6^{b}	3.1^{a}	2.8
LSD $p \leq 0.05$	ns			–

Statistically significant differences in means within groups (lines) are marked with letters, ± – standard deviation, substrates: A – soil (S) as a control object, B – soil + compost (S + C; 50 : 50), C – compost

Effect of substrates: substrate A (as control, soil only): consistently resulted in the lowest dry matter yield in all years, with an average of 2.31 t ha^{-1}. This indicates that soil alone provides limited nutrients and is less effective in supporting optimal plant growth. Substrate B (soil + compost, 50 : 50) showed higher yields, as compared to substrate A, with an average of 2.69 t ha^{-1}. This suggests that the addition of compost increases soil fertility and nutrient availability, promoting better growth. Finally, substrate C (compost only) gave the highest yield in all years, with an average of 3.25 t ha^{-1}. This highlights the higher effectiveness of compost in providing nutrients and creating favorable growth conditions. Year effect: in all substrates, the aboveground dry matter yield increased consistently over the three years, with average values of 2.5 t ha^{-1} (2021), 2.6 t ha^{-1} (2022) and 3.1 t ha^{-1} (2023). This may indicate an improvement in environmental conditions or an increase in the efficiency of experimental management. The LSD values ($p \leq 0.05$) for substrates and years (0.1) indicate that the differences between substrates and between years are statistically significant (tab. 7). This only strengthens the conclusion that both the choice of substrate and the year of cultivation have a significant effect on yield.

Interaction: consistent classification of substrate C > substrate B > substrate A in all years suggests that the effect of substrate type is strong and is not dependent on year-to-year changes. Thus, the obtained results of the study show that compost (substrate C) is the most effective substrate for obtaining high dry matter yields of ashwagandha, followed by soil and compost mixture (substrate B) – tab. 6. Soil alone (substrate A) gives the lowest results. The improvement in yields over the years suggests either better environmental conditions or adaptive management practices during the study period.

Table 7 illustrates the yield of fresh root mass of *V. somnifera* depending on the year and type of substrate. The standard deviation for yield of fresh mass of roots of ashwagandha (e.g. from 3.1 ±0.6 to 4.0 ±0.2) for a compost substrate) confirmed that the measurements were repeatable (tab. 7).

Table 7. Yield of fresh mass of roots of ashwagandha (t ha^{-1})

Substrates	Years			Mean
	2021	2022	2023	
A	3.1 ±0.3 ^{b}	3.1 ±0.4 ^{a}	3.0 ±0.3 ^{a}	3.1 ^{c}
B	3.5 ±0.3 ^{b}	3.1 ±0.2 ^{a}	3.0 ±0.4 ^{a}	3.2 ^{b}
C	4.0 ±0.2 ^{a}	3.5 ±0.2 ^{a}	3.1 ±0.6 ^{a}	3.5 ^{a}
LSD $p \leq 0.05$	0.5			2.37
Mean	3.5 ^{a}	3.2 ^{b}	3.0 ^{c}	3.3
LSD $p \leq 0.05$	ns			–

Statistically significant differences in means within groups (lines) are marked with letters, ± – standard deviation, substrates: A – soil (S) as a control object, B – soil + compost (S+C) (50 : 50), C – compost

The data obtained indicate that the use of compost, either alone (substrate C) or mixed with soil (substrate B), increases the yield of ashwagandha root fresh weight compared to soil alone (substrate A). Over the three years, substrate C consistently produced the highest yield, averaging 3.54 t ha^{-1}, followed by substrate B with 3.19 t ha^{-1} and substrate A with 3.07 t ha^{-1}. The least significant difference (LSD) at $p \leq 0.05$ for the mean values is 0.17, indicating that the differences between substrates C and A, as well as between C and B, are statistically significant, whereas there is no difference between substrates A and B. This suggests that the inclusion of compost in the growing medium can significantly improve the yield of ashwagandha root (tab. 7). These results are consistent with previous studies showing that organic nutrient sources such as compost positively affect the yield and quality of ashwagandha. For example, a field experiment investigating the effect of organic nutrient management on ashwagandha showed increased yield and quality parameters with the use of organic amendments.

Analysis of the interaction between years and substrate types in relation to the yield of fresh root mass of *Withania somnifera* indicates significant differences in performance depending on the substrate used and the year of cultivation. A (control object): Fresh root yields were relatively stable over the years of the study, with a slight decrease from 3.1 t ha^{-1} in 2021 to 3.0 t ha^{-1} in 2023. Substrate B (soil + compost in a ratio of 50 : 50), where a decrease in yields was observed from 3.5 t ha^{-1} in 2021 to 3.0 t ha^{-1} in 2023, which suggests a decreasing efficiency of this substrate in the following years. Substrate C (compost), where the highest yields were obtained in this substrate, from 4.0 t ha^{-1} in 2021, however, a decrease in fresh root yield was observed to 3.1 t ha^{-1} in 2023 (tab. 7).

In 2021 the highest yields were obtained in substrate C (4.00 t ha^{-1}) and the lowest in substrate A (3.1 t ha^{-1}). In 2022, substrate C continued to outperform the others (3.51 t ha^{-1}), while substrate B had a slightly higher yield (3.1 t ha^{-1}) than substrate A (3.1 t ha^{-1}). In 2023, although substrate C maintained the highest yield (3.1 t ha^{-1}), the differences between substrates were smaller and all substrates showed a decreasing trend compared to previous years. Summary: Substrate C (compost) showed the highest efficiency in increasing ashwagandha root fresh mass yields in all years, but a decreasing trend is noticeable over time. Substrate B (soil + compost) did not bring the expected benefits compared to substrate A (control soil) and even showed a decrease in yields in 2023 (tab. 7). The overall downward trend in yields from 2021 to 2023 suggests the possibility of substrate nutrient depletion or other environmental factors, which require further investigation. Crop rotation, substrate regeneration, or additional fertilization may also be considered to maintain high yields over the longer term (tab. 7). Table 8 illustrates the influence of substrates and years on the yield of dry root mass of *Withania somnifera*.

Table 8. Yield of dry mass of ashwagandha roots (t ha^{-1})

Substrates	Years			Mean
	2021	2022	2023	
A	1.4 ±0.1 ^{b}	1.3 ±0.1 ^{b}	1.2 ±0.1 ^{b}	1.3 ^{c}
B	1.5 ±0.1 ^{a}	1.4 ±0.1 ^{a}	1.3 ±0.2 ^{a}	1.4 ^{b}
C	1.8 ±0.3 ^{a}	1.5 ±0.2 ^{a}	1.4 ±0.1 ^{a}	1.6 ^{a}
LSD $p \leq 0.05$	0.2			0.1
Mean	1.6 ^{a}	1.4 ^{b}	1.3 ^{c}	1.4
LSD $p \leq 0.05$	0.1			–

Statistically significant differences in means within groups (lines) are marked with letters, ± – standard deviation, substrates: A – soil (S) as a control object; B – soil + compost (S+C) (50 : 50); C – compost

Table 8 data shows that substrate composition significantly affects the root dry matter yield of ashwagandha. Over the three-year period (2021–2023), substrate C (compost) consistently produced the highest mean root dry matter yield of 1.55 t ha^{-1}, followed by substrate B (soil + compost) with 1.4 t ha^{-1} and substrate A (soil) with 1.3 t ha^{-1}. The least significant difference at $p \leq 0.05$ was 0.1, suggesting that the differences between these means are statistically significant. This trend was consistent across all three years of the study, with substrate C significantly outperforming the others, indicating that the use of compost as a substrate increases the root dry matter yield of ashwagandha. The interaction between years and substrates shows that although there are some differences in yields between years, the superiority of the C substrate remains consistent, highlighting the beneficial effect of compost on root biomass production (tab. 8). The standard deviation for yield of dry mass of ashwagandha roots (e.g. from 1.4 ±0.1 to 1.8 ±0.3) for a compost substrate and for an object B soil + compost (S + C, 50 : 50; e.g. from 1.31 ±0.2 to 1.5 ±0.1) confirmed that the measurements were repeatable (tab. 8).

These results are consistent with previous studies indicating that organic nutrient sources such as compost positively affect the yield and quality of ashwagandha. A field experiment evaluating the effect of organic nutrient management on ashwagandha showed increased yield values with the use of organic amendments. In conclusion, the incorporation of compost into the growing medium can significantly increase the root dry matter yield of ashwagandha, offering a viable strategy for improving crop productivity in sustainable agricultural practices.

DISCUSSION

Our research provides a comprehensive assessment of the factors influencing the cultivation of *Withania somnifera* in a temperate climate, with particular emphasis on the conditions prevailing in south-eastern Poland. The results obtained from both field and laboratory experiments are consistent and confirm that both biotic factors (soil quality) and abiotic factors (temperature) play a key role in determining the success of cultivation.

Energy and germination of seeds

Germination capacity and germination energy are critical determinants of early plant development and play a decisive role in crop productivity [Kucera et al. 2005, Nonaka et al. 2010, Faligowska and Szukała 2012, Faligowska et al. 2018, Genze et al. 2020, Mohamed et al. 2021]. These parameters directly influence seedling uniformity, vigor, and the ability to adapt to field conditions. As reported by Mohamed et al. [2021] and Rymuza and Radka [2021], seeds exhibiting high germination energy produce robust and uniform seedlings, which is essential for successful crop establishment and subsequent yield potential. Therefore, optimizing germination conditions is a fundamental aspect of agronomic management.

Our results indicate that a temperature of 30°C is optimal for both germination capacity and germination energy of *W. somnifera* seeds, yielding the highest recorded values of 95.45% and 93.56%, respectively (tab. 3 and 4). These findings are consistent with existing literature, which classifies *W. somnifera* as a thermophilic species that thrives in the warmer conditions characteristic of subtropical climates. Maintaining optimal temperature during germination is therefore essential to ensure uniform seedling emergence and vigorous early growth.

The germination process involves a series of complex physiological and biochemical changes, beginning with water uptake, which activates hydrolytic enzymes responsible for mobilizing stored nutrient reserves, and culminating in the emergence of the embryonic radicle, indicating the breaking of the seed coat [Kambizi et al. 2006, Faligowska and Szukała 2012, Faligowska et al. 2018, Mohamed et al. 2021, Rymuza and Radka 2021, Khaim et al. 2022, Kumar et al. 2022, Sharma et al. 2023]. Enzymes such as α-amylase and proteases are particularly critical, facilitating the conversion of starches and proteins into soluble forms that fuel embryonic growth [Xia et al. 2024]. Suboptimal temperatures can suppress enzymatic activity, leading to delayed or incomplete germination. Our observations indicate that temperatures below 20°C markedly reduce germination rates, while exposure to 10°C nearly inhibits germination entirely.

Conversely, high temperatures (~30°C) likely stimulate the synthesis of heat shock proteins (HSPs), which protect cellular structures and enzymes from thermal stress, thereby enhancing germination efficiency and producing uniform seedlings [Faligowska and Szukała 2012, Porter et al. 2016, Faligowska et al. 2018]. Unlike the findings of Kumar et al. [2016], who observed high germination under fluctuating temperatures (15/35°C), our study focused on constant temperature conditions, providing a clearer understanding of the thermal optimum for *W. somnifera*. These results complement existing literature, confirming that stable high temperatures are critical for successful germination of this species.

For cultivation in temperate climates, where early spring temperatures are often suboptimal, producing seedlings under controlled conditions, such as polytunnels or greenhouses maintained at approximately 30°C, is essential to ensure uniform emergence and robust early growth. Seedling vigor at this stage is particularly important, as it affects the plant's capacity to compete for resources and withstand environmental stresses in the field.

Future research should explore thermo-priming techniques, which involve preliminary thermal conditioning of seeds to enhance seedling tolerance to low temperatures, potentially extending the cultivation range of *W. somnifera* [Lim et al. 2015]. Additionally, the identification and selection of genotypes with superior adaptability to variable climatic conditions represent a promising strategy for improving crop resilience and ensuring sustainable production under changing environmental conditions [Singh et al. 2024].

The effect of substrate and environmental conditions on yield

Our research demonstrates that substrate type is the primary factor influencing the yield of both above-ground and root parts of *W. somnifera*. The highest fresh (14.36 t ha^{-1}) and dry matter (3.25 t ha^{-1}) yields from above-ground parts were obtained on substrate C (pure compost), which can be directly attributed to its high fertility [Patel and Rank 2022]. Physicochemical analysis (tab. 1) confirmed that substrate C had the highest concentrations of essential macronutrients (P_2O_5, K_2O, Mg) and micronutrients (Zn, Fe, Mn), along with a neutral pH of 7.10. These characteristics are known to favor optimal nutrient uptake and plant development. This finding aligns with previous studies highlighting the critical role of soil pH and nutrient availability in determining *W. somnifera* growth and productivity [Helfenstein et al. 2016, Neina 2019, Xia et al. 2024].

Soil pH plays a crucial role in controlling nutrient bioavailability [Lim et al. 2015], particularly for phosphorus and zinc, which are essential for enzymatic activity, energy transfer, and overall metabolic processes in plants. Acidic conditions, such as those observed in the control substrate (A) with a pH of 5.69, limit nutrient availability and consequently reduce both vegetative growth and yield [Singh et al. 2024]. Over the period 2021–2023, an increase in yield was observed on all substrates, likely reflecting a combination of improved meteorological conditions and progressive plant adaptation to the local environment, underscoring the interactive effects of edaphic and climatic factors on crop performance.

The highest fresh (3.54 t ha^{-1}) and dry root mass (1.55 t ha^{-1}) were also recorded on substrate C, confirming the exceptional nutrient-retention and fertility properties of high-quality compost [Patel and Rank 2022]. These results are consistent with numerous studies demonstrating that organic nutrient sources, such as compost and manure, significantly enhance both biomass accumulation and secondary metabolite content in *W. somnifera* [Patel and Rank 2022, Singh et al. 2024]. The availability of organic matter not only provides essential nutrients but also improves soil structure, water-holding capacity, and microbial activity, all of which contribute to improved root development and plant vigor.

Interestingly, while above-ground yields generally increased, root yields exhibited a downward trend across all substrates over the years 2021–2023. This observation cannot be fully explained by meteorological conditions alone, which were relatively favorable, particularly in 2023. Potential factors contributing to the decline in root biomass include:

Nutrient depletion: despite the initially high fertility of the substrates, continuous cultivation without adequate substrate regeneration may have gradually reduced nutrient availability, particularly in the root zone.

Lack of crop rotation: Monoculture practices can lead to the accumulation of soil-borne pathogens, negatively impacting root development and overall plant health [Noworolnik 2015, Helfenstein et al. 2016, Porter et al. 2016, Neina 2019, Xia et al. 2024].

These findings emphasize that even the most fertile organic substrates require a comprehensive, long-term management strategy. Substrate regeneration, rotational practices, and supplementary fertilization are necessary to maintain soil fertility and sustain high yields over multiple growing seasons. This highlights an important practical consideration often overlooked in single-season studies, underlining the need for long-term experiments to fully understand substrate effects on crop productivity.

Furthermore, these results have implications for optimizing cultivation protocols for *W. somnifera*, especially in regions with marginal soils or limited access to high-quality compost. Integrating organic amendments with targeted nutrient management and adaptive agronomic practices can enhance both above-ground biomass and root yield, contributing to sustainable production systems and maximizing the medicinal potential of this species.

CONCLUSIONS

The results of the study confirmed hypothesis H_1, indicating that temperature had a statistically significant effect on the germination capacity of *W. somnifera* seeds. The null hypothesis H_{01} was therefore rejected. Similarly, the obtained results confirmed hypothesis H_2, showing that different substrate types had a significant effect on the yield of *W. somnifera*. Consequently, the null hypothesis H_{02} was also rejected.

The study clearly demonstrated that both germination temperature and substrate type play a crucial role in the successful cultivation of *Withania somnifera* L. The optimal germination temperature was found to be 30°C, at which the highest germination capacity and energy (over 90%) were recorded, confirming the plant's strong preference for warm, subtropical conditions. Lower temperatures, especially 10°C, almost completely inhibited effective germination, ruling out the possibility of cultivation in cooler climates without temperature control.

The composition of the substrate was equally important – the highest yields of both aerial parts and roots were obtained on a substrate consisting exclusively of compost (substrate C), indicating the high fertilization value of organic matter and its positive effect on the physical and chemical properties of the soil. Although a slight decrease in yields was observed in the third year of the study, the results confirmed that compost significantly improves both the quality and quantity of ashwagandha production.

In the context of sustainable agriculture and the growing demand for medicinal plant raw materials, it is recommended to implement practices such as substrate rotation, regeneration, and further optimization of organic fertilization. Future efforts should focus on maintaining high productivity while ensuring the quality of raw materials and the long-term sustainability of cultivation.

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