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Pietro Romualdo Pirotta, founder, 1884

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OPTIMIZING GERMINATION AND DETERMINING PHYSIOLOGICAL MATURITY OF *RUBUS FRAXINIFOLIUS* SEEDS FOR STANDARDIZED SEED TESTING PROTOCOLS

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Abstract - *Rubus fraxinifolius*, a species of *Rubus* found in Indonesia, is an edible plant with high value and significant potential for domestication as a fruit crop. The requirements for seed germination are integral to the domestication process and necessitate improvement and standardization for *R. fraxinifolius* to produce high-quality seeds for breeding programs. Seed quality reaches maximum physiological maturity and is characterized by seed germination, vigor, and moisture content. This study aimed to enhance germination tests and determine the quality of *R. fraxinifolius* seeds. Two experiments were conducted, comprising seed germination testing and determination of seed physiological maturity. *R. fraxinifolius* seed germination demonstrated optimal results when seeds were treated with 1000 ppm KNO_3 using pleated paper, yielding a germination percentage of 82.5%, vigor index of 27.5%, and speed of germination of 1.94%/day. Physiological maturity was attained 39-40 days after anthesis, from fully ripe fruits with a red color that readily detached from the pedicle, exhibiting a maximum seed dry weight of 0.194 g, germination percentage of 95.8%, and vigor index of 71.6%. These germination test protocols were subsequently employed to evaluate the quality of *R. fraxinifolius* seeds in the breeding program and overcome seeds dormancy, potentially resulting in improved germination outcomes.

Keywords: final count, first count, germination protocols, pleated paper, top of paper, wild-type raspberry.

Introduction

Commercial Rubus fruits, such as raspberries (*Rubus idaeus*), blackberries (*Rubus* sp.), and dwarf raspberries (*Rubus pubescens*), hold significant value in several countries, including the United States, Russia, China, and Japan. However, there is limited information regarding the cultivation and utilization of Rubus species in Indonesia. Despite their presence in various regions such as West Java, Kalimantan, and the mountainous forests of Sulawesi, the cultivation and commercialization of these fruits are not yet widespread (Normasiwi et al., 2021). In Cianjur, West Java, *R. fraxinifolius* is native species known locally as "arben", has not only gained popularity but also become a part of the local community's identity (Surya et al., 2018). This local recognition and acceptance of *R. fraxinifolius* present a promising opportunity for its domestication and commercialization as a commercial fruit crop (Normasiwi et al., 2021).

The reproductive biology of the species to be domesticated is crucial for comprehending the mode of pollination, the necessity for pollinators, fruit development and maturation patterns, and the requirements for seed germination (Fredes et al., 2016). Although cultivars of raspberries are propagated clonally, significant traits for improvement through breeding programs are present in wild germplasm, which is accessed from stored seeds of wild-collected plants (Wada & Reed, 2011b). One of the challenges in *Rubus* breeding is the exceptionally slow and irregular germination of seeds and the limited number of seedlings obtained, as protocols for

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germination of wild species are not readily available (Wada & Reed, 2011b; Wada et al., 2011; Żurawicz et al., 2017).

Physiological maturity is a crucial element for the production of high-quality seeds, which is characterized by the highest seed dry weight because the accumulation of storage substances reaches its peak and vigor is at its highest, and the seed moisture content begins to decrease (Ilyas, 2012). During histodifferentiation and cell division, fresh weight and seed moisture content increase and then decrease in parallel with the increase in dry weight. Seed drying occurs during the ripening stage (Bewley & Black, 1994). The seeds should be harvested at physiological maturity, as their viability and vigor will decrease if left in the field after this stage (Suharsi et al., 2015). Fruit maturity is one of the methods that can be used to determine the physiological maturity of seeds (Aminah & Siregar, 2019). This involves assessing the fruit color, smell, hardness of the fruit or seed skin, fruit or seed loss, and fruit rupture (Rusmin & Darwati, 2018), which are all benchmarks associated with physiological seed quality in the seed germination test.

Rubus seed germination is constrained by physical, physiological, or both factors (Wada & Reed, 2011a). The raspberry seed germination problem was not only the scarification and stratification method, but also the varied response of seeds of different species to seed pre-treatment before sowing (Clark et al., 2007). According to Żurawicz et al. (2017), the best germinating raspberry seeds were treated with sulfuric acid for 30 min. Wada & Reed (2011b) recommended that *Rubus* seeds would be scarified with H_2SO_4 and stratified by treatment with 34 ppm GA_3+KNO_3. However, during seed treatment with acid chemical material, the seed coat can be damaged, as can the seed embryo (Żurawicz et al., 2017). However, since there were no protocols for germination and dormancy breaking of *R. fraxinifolius*, this study used modification of germination protocols for other *Rubus* species by Wada & Reed (2011b) and ISTA (2018).

The main goals of this study were to establish criteria for seedlings, as well as to identify the optimal germination method and seed treatment for faster germination. These findings will be valuable in setting seed testing protocols because no such information is currently available for *R. fraxinifolius* seeds in the ISTA reference. These germination test protocols were then used to evaluate the physiological maturity of *R. fraxinifolius* seeds to produce high-quality seeds.

Materials and Methods

The experiment was undertaken at the Cibodas Botanical Garden and Seed Health Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University between January and July 2023. The Cibodas Botanical Garden where *R. fraxinifolius* is cultivated is at an elevation of 1300 masl, with an average temperature of 24.43 ± 0.44 °C, an average humidity of 88.98 ± 1.69%, and a daily rainfall of 20.48 mm (NASA, 2023).

Germination method and treatment soaking seeds determination

The seeds were obtained from *R. fraxinifolius* fruits that were harvested at 40 days after anthesis (DAA) in January 2023. These fruits were considered morphologically ripe due to their full red color and ability to detach easily from the pedicle (Perkins-Veazie & Nonnecke, 1992; Fuentes et al., 2019). The study was designed as a two-factor factorial experiment with a completely randomized design. The first factor consisted of two germination test methods: top of paper (TP) and pleated paper (PP). The second factor was a seed-soaking treatment, consisted of control, distilled water, 30 ppm KNO_3, 1000 ppm KNO_3, 5 ppm GA_3, 100 ppm GA_3, and a mixture of 30 ppm KNO_3 and 5 ppm GA_3. Chemical KNO_3 and GA_3 used from Merck KGaA, Germany. There were 14 treatment combinations in total, and each was repeated four times with 50 seeds per replication.

The seed extraction involved carefully separating the seeds from the fruit and rinsing them with clean tap water to eliminate any residual pulp. Subsequently, the seeds were desiccated at room temperature for five days to prevent mould growth and ensure optimal condition for storage or planting (Wada & Reed, 2011b). The seeds were subsequently immersed in a seed-soaking treatment solution for 24 h at an incubation temperature of 20 ± 2°C before sowing. The top of paper (TP) method utilized three sheets of filter paper with a piece of tissue as the base, while the pleated paper (PP) method employed a folded filter paper with each fold measuring 2 cm in width. The filter paper that utilized was composed of cellulose cotton material with a thickness of 200 ± 10 µm and an ash content of 0.15%. Distilled water was meticulously applied to moisten the media. The germination process was conducted in a plastic container with dimensions of 8 cm × 8 cm × 5 cm, using a room germinator at a temperature of 20 ± 2°C.

The daily counts of normal seedlings were recorded and formed into a curve to determine the initial and final germination counts. The frequency of normal seedlings increased progressively until they reached their peak, which served as the first count for the evaluation of germination. The germination percentage was determined based on the rapidity of the peaks. The final count was established when the graph of seedling accumulation exhibited a slope, indicating that few or no seeds germinated. The inclusion of a small number of normal seedlings per day results in a sloping and stationary graph (Sadjad, 1994). The interaction of factors facilitating the germination of *R. fraxinifolius* was considered for the initial and final count determination with the *R. fraxinifolius* seed germination test, consisting of:

a. Germination percentage (GP) was carried out on normal seedlings

$$GP\ (\%) = \frac{\sum \text{Normal Seedling in first count} + \text{Normal Seedling in final count}}{\text{number of seeds tested}} \times 100\%$$

b. Speed of germination (GS) was calculated based on the sum of normal seedlings every day during the testing period.

$$GS\ (\%/\text{day}) = \sum_{i=1}^{n} \frac{\%\ \text{Normal seedlings at day i}}{n}$$

c. The vigor index (VI) was calculated based on the number of normal seedlings that appeared in the first count.

$$VI\ (\%) = \frac{\text{Normal Seedling in first count}}{\text{number of seeds tested}} \times 100\%$$

Physiological maturity determination of *R. fraxinifolius* seeds

Randomized complete block design (RCBD) with a single factor, comprising six different fruit harvesting times, consisted of 35, 36, 37, 38, 39, and 40 DAA fruits. Each treatment was divided into four groups, with each replicate containing 50 seeds. The experiment commenced with the labeling of flowers that had not fully reached anthesis. The fruit color was assessed using the RHS color chart (RHS, 2015). The seed processing, germination method, and seed treatment were based on the optimal results obtained in the previous experiment. The collected seed was extracted and dried for five days at room temperature (Wada & Reed, 2011b). The seeds were immersed in 1000 ppm KNO_3 for 24 h at an incubation temperature of 20 ± 2°C before sowed. The germination was conducted in a plastic box with pleated paper method, and incubated in a room germinator at a temperature of 20 ± 2°C.
The viability of *R. fraxinifolius* seeds was assessed based on two variables: germination percentage (GP) and maximum growth potential (MGP). The physiological quality variables observed in *R. fraxinifolius* seed for physiological maturity determination were as follows:

a. Seed dry weight (g) was measured according to the procedures outlined in ISTA (2018) by weighing seeds in each replicate and drying them using the oven method at 80°C for 24 h. The seeds were then placed in a desiccator and weighed.

b. Moisture content (%) was determined using the direct method (oven) at 103 ± 2 °C for 17 ± 1 h. The seed sample used was ± 0.25 g. After being oven dried, the seeds were placed in a desiccator for 30 minutes and weighed (ISTA, 2018).

c. Maximum growth potential (MGP) was determined based on the percentage of seedlings at the end of observation (Sadjad, 1994):

$$MGP\ (\%) = \frac{\sum NS1 + NS2 + ANS1 + ANS2}{\text{number of seeds tested}} \times 100\%$$

NS1= normal seedlings in the first count; NS2= normal seedlings in the final count; ANS1= abnormal seedlings in the first count; ANS2= abnormal seedlings in the final count.

Data Analysis

Data were analyzed using ANOVA, and Duncan's multiple range test (DMRT) (α = 0,05) was used for further statistical analysis if there was a significant difference. tests were carried out using the Agricolae package (de Mendiburu, 2020) in R-Studio version 2022.02.3. Microsoft Excel 2019 and CurveExpert version 1.3 was also used for statistical analysis and to construct the curve.

Results

The structure of *R. fraxinifolius* seedlings are shown in Fig. 1.

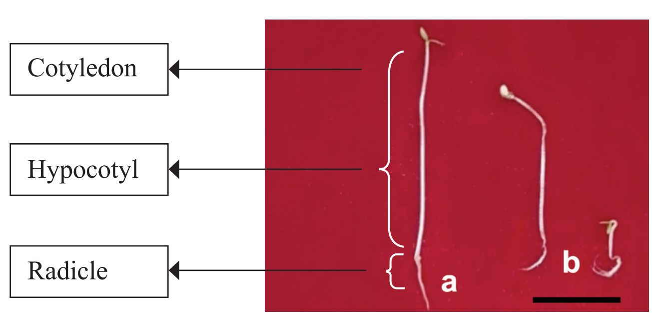


Figure 1. Structure of *R. fraxinifolius* seedlings (a) Normal seedling; (b) Abnormal seedling. Scale= 1 cm

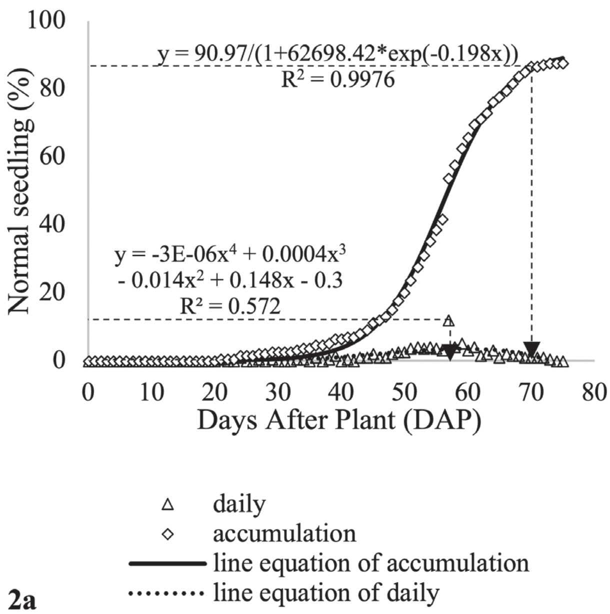


Figure 2a. Determination curve of *R. fraxinifolius* germination by soaking in 1000 ppm KNO_3 under TP method.

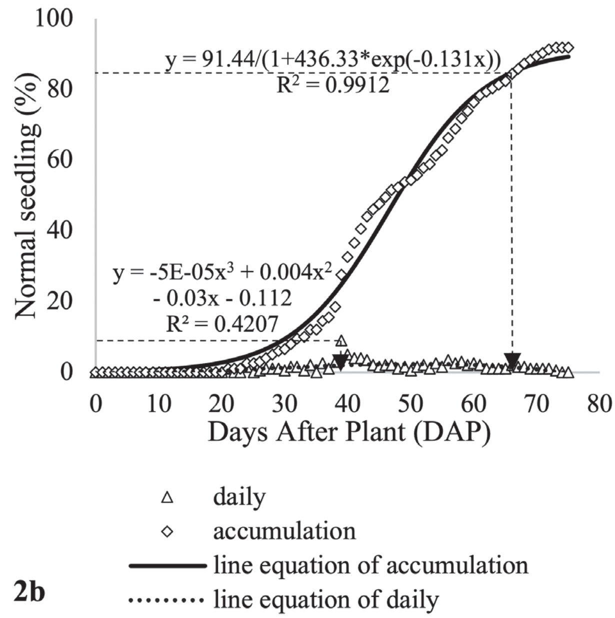


Figure 2b. Determination curve of *R. fraxinifolius* germination by soaking in 1000 ppm KNO_3 under PP method.

A healthy radicle with a minimum length of 0.5 cm, a straight hypocotyl of at least 2 cm, and a detached seed coat from the cotyledon (a) were indicative of normal seedling development. Abnormal seedlings of raspberry seeds were characterized by structures that were unable to exhibit normal growth symptoms, including short radicles, damaged, rotten, or coiled hypocotyls, and a seed coat still attached to the cotyledon (b).

Figure 2a and 2b illustrates the initial and final count determinations. The TP method, incorporating seed soaking treatment, achieved its peak at 57-59 days after planting (DAP). The application of 1000 ppm KNO_3 as a soaking treatment in the TP method resulted in the most rapid peak of normal seedling emergence at 57 DAP (Figure 2a).
The treatment involving 1000 ppm KNO_3 immersion in the PP method can serve as a benchmark for determining the

Table 1. Average seed quality variables of *R. fraxinifolius* based on germination method and seed soaking treatment.

Seed treatment	Germination method					
	PP	TP	PP	TP	PP	TP
	-----GP (%)-----		-----VI (%)-----		----GS (%/day)----	
Control	60.5^{cB}	71.0^{aA}	2.0^{cA}	0.5^{cB}	1.09^{cA}	1.23^{bA}
Distilled Water	69.0^{abcA}	75.5^{aA}	13.0^{bA}	1.5^{bcB}	1.45^{bA}	1.35^{abA}
30 ppm KNO_3	76.5^{abA}	71.0^{aA}	10.5^{bA}	2.5^{bB}	1.58^{bA}	1.30^{bA}
1000 ppm KNO_3	82.5^{aA}	77.5^{aA}	27.5^{aA}	6.5^{aB}	1.94^{aA}	1.51^{aB}
5 ppm GA_3	65.0^{bcA}	78.2^{aA}	20.0^{aA}	0.0^{cB}	1.46^{bA}	1.41^{abA}
100 ppm GA_3	73.0^{abcA}	68.5^{aA}	9.0^{bA}	1.0^{bcB}	1.49^{bA}	1.24^{bA}
5 ppm GA_3 + 30 ppm KNO_3	73.0^{abcA}	74.0^{aA}	25.5^{aA}	0.5^{cB}	1.74^{abA}	1.33^{abB}

Numbers followed by the same letter within a column or row indicate no significant difference based on DMRT at $\alpha = 5\%$. Capital letters in each row for the variables GP, VI, and GS represent the effect of the germination method (PP and TP), while lowercase letters in each column indicate the effect of seed treatment. PP=pleated paper; TP=top of paper; GP=germination percentage; VI=vigor index; GS=speed of germination.

initial and final counts. The increase in normal seedlings begins to decline, producing a sloping graph after 65 DAP, indicating that the final germination count can be established at 65 DAP (Fig. 2a). In contrast, the use of 1000 ppm KNO_3 in the PP method reached its peak at 39 DAP (Fig. 2b).

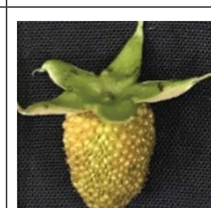
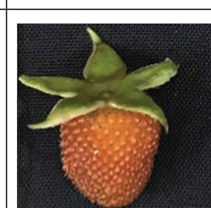
Germination method and pre-treatment soaking seeds determination

The germination method of *R. faxinifolius* did not have a substantial influence on the GP variable, but did have a significant effect on the VI and GS variables. Additionally, the seed soaking treatment factor had a singular impact on all three germination evaluation variables. Furthermore, the factors of germination method and seed soaking treatment demonstrated a significant interaction in their effect on VI and GS (as shown in Table 1).
The influence of seed soaking and germination methods on seed viability and vigor is significant. Seed viability can be evaluated by GP and MPG, while seed vigor by GS and VI. In the same seed treatment (control), PP method resulted in a GP of 60.5%, while the TP method had a GP of 71.0%. The interaction between the PP method and seed soaking treatment with 1000 ppm KNO_3 proved to be the most effective among the various combinations evaluated, as evidenced by the highest VI (27.5%) and GS (1.94%/day).

Physiological maturity determination of *R. fraxinifolius* seeds

The physiological maturity determination of *R. fraxinifolius* seeds was accomplished using an approach based on fruit maturity and harvest time. Fruits that were 25 DAA (Days After Anthesis) in age were still green and hard, and began to change color at 30 DAA (Table 2). There were visible changes occurred in both color and fruit hardness from 35 to 40 DAA, the fruit size increased until it detached from the torus. The physiological maturity of *R. fraxinifolius* seeds was determined at 35-40 DAA, with differences in fruit color being evident as shown in Table 2. Two physical attributes of seeds as benchmarks for assessing physiological maturity are seed moisture content and seed

Table 2. *R. fraxinifolius* fruit color at various harvest times.

Harvest time (DAA)	Fruit color		Color code (RHS)	Figure of fruit
25	Green Group	Strong Yellow Green	143 A	
30	Yellow-Green Group	Strong Yellow Green	144 A	
35	Yellow-Green Group	Strong Greenish Yellow	151 A	
36	Orange Group	Strong Orange	N 25 B	

Harvest time (DAA)	Fruit color		Color code (RHS)	Figure of fruit
37	Red Group	Vivid Red	44 A	
38	Red Group	Vivid Red	45 A	
39	Red Group	Vivid Reddish Orange	43 A	
40	Red Group	Vivid Reddish Orange	43 A	

DAA= Days After Anthesis; RHS = Royal Horticultural Society; Scale= 1 cm

dry weight. According to Table 3, the moisture content of *R. fraxinifolius* seeds exhibited a significant decline between 35 and 37 days after anthesis (DAA), followed by a gradual decrease that was not statistically significant until 40 DAA. This reduction in seed moisture content contrasted with the increase in seed dry weight (SDW) observed during seed maturation. The highest SDW value was recorded at 40 DAA, reaching 0.194 g, and was significantly influenced by other harvest times. This finding indicates the attainment of maximum SDW in seeds harvested at 40 DAA.

Rubus fraxinifolius seeds were found to be capable of germination at any time of fruit harvesting, but there were differences in GP value. Fruit harvested at 40 DAA produced seeds with the highest GP at 95.8%, but this did not have a significant effect on the GP at 39 DAA, which was 94.5%. The MGP value of seeds harvested at 38 DAA was not significantly different from the seeds harvested at 39 and 40 DAA, which was 93.8%. On the other hand, fruit harvested at 35 DAA had the lowest GP at 87.6%, and was significantly different compared to other fruit ages, in accordance with MGP value. The highest VI of *R. fraxinifolius* seeds was obtained at 40 DAA, but did not have a significantly difference compared to 39 DAA, about 71.6% (Table 4).

Discussion

The criteria for defining normal *R. fraxinifolius* seedlings were established by assessing the key structures of the radicle, hypocotyl, and cotyledons. The emergence of normal *R. fraxinifolius* seedlings occurred between 21 and 24 DAP. The first and final counts are essential for

Table 3. Moisture content and dry weight of *R. fraxinifolius* seeds based on different harvesting time

Harvest time (DAA)	Seed moisture content (%)	Seed dry weight (g)
35	35.9[a]	0.181[d]
36	34.5[b]	0.184[cd]
37	33.5[c]	0.185[bcd]
38	33.3[c]	0.188[bc]
39	33.3[c]	0.189[b]
40	32.8[c]	0.194[a]

Numbers followed by the same letter in the same column indicate no significant effect in the DMRT test $\alpha = 5\%$. DAA= Days After Anthesis.

Table 4. Viability of *R. fraxinifolius* seeds based on different harvesting time

Harvest time (DAA)	Germination percentage (%)	Maximum growth potential (%)	Vigor index (%)	Speed of germination (%/day)
35	87.6[d]	89.1[d]	42.1[d]	2.20[e]
36	91.0[c]	92.2[c]	49.4[d]	2.35[d]
37	92.2[bc]	92.9[bc]	59.5[c]	2.47[c]
38	93.0[bc]	93.8[abc]	63.1[bc]	2.52[bc]
39	94.5[ab]	95.2[ab]	67.9[ab]	2.62[ab]
40	95.8[a]	96.0[a]	71.6[a]	2.67[a]

Numbers followed by the same letter in the same column indicate no significant effect in the DMRT test $\alpha = 5\%$. DAA= Days After Anthesis.

evaluating seed germination. The observation of the first count serves as a test of seed vigor, enabling the assessment of the germination rate.

Germination method and pre-treatment soaking seeds determination

The pleated paper germination method is a widely utilized technique for germinating small seeds. It has been observed that when using the top of paper method for the germination of *Silphium perfoliatum* L., fungal infection occurs. However, the pleated paper method, due to its folded structure, serves as a barrier that prevents the spread of fungi from one seed to another (Bareke, 2018). The paper medium used in this method should possess exceptional moisture absorption and retention capabilities, as well as display capillary rise potential, maintain a neutral pH, and be free from any phytotoxic effects (Santos et al., 2022).

The germination phase is significantly influenced by hormonal regulation, particularly abscisic acid (ABA) and gibberellin acid (GA_3). ABA inhibits germination, while gibberellin supports it (De Wit et al., 2016). ABA accumulates in the embryo during seed maturation and plays a crucial role in primary dormancy, while GA_3 induces embryo growth and increases nutrient availability in the seed (Gansberger et al., 2017; Bareke, 2018). They promote growth by increasing cell wall plasticity, followed by the hydrolysis of starch into sugars that reduce cell potential pressure, allowing water to enter the cell for germination (Gupta & Chakrabarty, 2013; Agurahe et al., 2019). Potassium nitrate (KNO_3) is a nitrogen compound that promotes germination and breaks seed dormancy in various plants. The impact of KNO_3 is contingent upon the concentration utilized and the specific type of plant being tested (Bareke, 2018).

The germination percentage below 80% suggests a state seed dormancy. *Rubus* seeds exhibit both physical and physiological dormancy, as reported by Wada & Reed (2011b). The seed coat of *Rubus* and other Rosaceae species is impermeable to both water and gas. Hydrogen cyanide (HCN) is formed when water uptake reaches inhibitory levels and remains until the seed coat and endosperm pellicle are either damaged or partially removed, as indicated by Wada et al. (2011b). The permeability of the seed coat to water is influenced by the seed coat's phenolic content and its degree of oxidation, as stated by Marbach & Mayer (1974). It is crucial to administer a dormancy-breaking treatment to *R. fraxinifolius* seeds in order to eliminate any compounds that inhibit germination and improve the seed coat's permeability.

The folds in the PP method prevent light from reaching smaller seeds, thereby inhibiting germination. Gansberger et al. (2017) found that the germination percentage of the PP method was similar to that of the TP method under minimal light conditions. Light is a regulator of plant growth and development, from the germination to the aging phase (Warpeha & Montgomery, 2016; Deepika et al., 2020). Hormones can replace the role of light by stimulating germination enzymes (Kołodziejek, 2017).

The vigor index signifies the proportion of seeds that germinate at first count and the PP method typically yields the highest VI. The TP method exhibits a low VI due to its extended peak of normal germination. This disparity is linked to the GS, reflecting that certain treatments in the PP method are more expeditious than TP method. The folds on PP method may possess better water-storing capabilities, maintaining the humidity of the germination environment, and sustaining germination over an extended period. In the study of Gundala et al. (2023), the small sized Sambiloto (*Andrographis paniculata*) seeds germinated twice as fast in the PP method, as they are enveloped by moist paper, resulting in a slower evaporation of water. ISTA (2018) also recommends pleated paper for small seeds, like *R. fraxinifolius* seed. The hard seed coat of *R. fraxinifolius* necessitates high humidity for germination. According to Baskin & Baskin (2004), light and substrate moisture serve as two essential dormancy-breaking factors that facilitate seed germination.

Determining the optimal combination of treatment and germination method is a challenging task when relying on the GP value, as the resulting value lacks significant practical implications. Based on the three germination variables examined, it is concluded that the combination of 1000 ppm KNO_3 soaking treatment with the PP method is the most effective treatment for *R. fraxinifolius* germination percentage. The application of KNO_3 is known to involve hydrogen receptors such as nitrate, which play a role in the reoxidation process of NADPH, a coenzyme that facilitates the chemical reactions and respiration required for seed germination (Bareke, 2018).

Physiological maturity determination of *R. fraxinifolius* seeds

Wet or fleshy fruit, such as those of *R. fraxinifolius,* does not experience a sharp decline in moisture content like dry fruit (Marcos-Filho, 2016). *R. fraxinifolius* seeds belong to the orthodox seed group, characterized by a rapid increase in seed moisture content during the embryogenesis phase, where cell division and food reserves accumulation occur (Bonner, 1996). The decrease in moisture content is due to the release of water from cells as food reserves accumulate (Bradford, 1994). The increase in seed dry weight (SDW) of *R. fraxinifolius* with longer harvest time was observed. The end of seed development is reached when seeds physiological mature (Marcos-Filho, 2016).

The maximum growth potential of seeds refers to their ability to germinate both normally and abnormally. The higher the level of fruit ripeness, the greater the MGP value. The MGP value of seeds harvested at 38 DAA was not significantly different from the seeds harvested at 39 and 40 DAA. This suggests that seeds at 38 DAA had high viability, whereas at 35 DAA, the MGP value was low and significantly different from other harvest times. According to Kartika & Ilyas (1994), Jogo nut seeds do not germinate well at the beginning of the seed development stage due to insufficient food reserves to support germination. Copeland & McDonald (2001) state that seeds at physiological maturity have sufficient food reserves to support germination.

The vigor index, which represents the percentage of normal seedlings in the initial count, serves as an indicator of the swiftness with which seeds germinate (Sadjad et al., 1999). This research revealed that the highest VI of *R. fraxinifolius* seeds was obtained at 40 DAA, but did not demonstrate a significant difference compared to 39 DAA. Based on these results, it can be inferred that maximum vigor has been achieved 39 DAA. Additionally, the speed of germination (GS) variable is another measure of seed vigor. The highest GS value was observed in 40 DAA seeds, but not significantly different compared to 39 DAA seeds, about 2.67%/day. Seeds with high vigor are likely to grow faster than those with low vigor (Rori et al., 2018).

The values of GP, MGP, VI, and GS have reached their peak at 39 DAA during the germination of seeds, while seed dry weight only attained its maximum at 40 DAA. Therefore, the time of physiological maturity of seeds can be estimated to be around 39 and 40 DAA. It is recommended that seed harvesting be carried out shortly after the seeds reach physiological maturity and the moisture content has decreased. The optimal time for harvesting *R. fraxinifolius* seeds is at 40 DAA, when the physiological quality variables and dry weight have attained their maximum, and the moisture content has begun to decline. After 40 DAA, *R. fraxinifolius* fruit will fall by itself from the pedicle, so it cannot be detected which the fruit has recently or a long time fallen. Although determining physiological maturity by the level of fruit ripeness is a straightforward and simple method, it is subject to the influence of environmental factors that can affect the period of physiological maturity. High humidity and rainfall can cause flower and fruit development to be delayed, resulting in a shift in the physiological maturity of seeds (Normasiwi et al., 2021; Wlodarczyk et al., 2023). Another approach that can be employed to obtain measurable and objective results in determining physiological maturity is to quantify fruit color or calculate the accumulation of heat units in the fruit of *R. fraxinifolius* or other *Rubus* species.

Conclusions

Germination of *R. fraxinifolius* seeds can be initiated through treatment involving immersion in 1000 ppm KNO_3 for 24 hours, followed by germination using the pleated paper method on filter paper media. Incubation was conducted at a temperature of 25 ± 2°C, with the first count performed at 39 days after plant (DAP) and the final count at 65 DAP. Seeds harvested at 39-40 days after anthesis (DAA) exhibited optimal quality, including maximum dry weight, viability, and vigor. These seeds were physiologically mature, characterized by fully ripened fruits displaying complete red coloration and facile detachment from the pedicle. These findings can serve as preliminary information for developing methods to enhance germination and overcome dormancy in *R. fraxinifolius* seeds, potentially resulting in improved germination outcomes**.**

References

Agurahe, L., Rampe, H. L., & Mantiri, F. R. (2019). Pematahan dormansi benih pala (*Myristica Fragrans Houtt*.) menggunakan hormon giberalin. *Pharmacon, 8(*1), 30-40.

Aminah, A., & Siregar, N. (2019). Pengaruh waktu pengunduhan dan warna kulit buah terhadap daya berkecambah dan pertumbuhan bibit mindi (*Melia azedarach Linn*). *Jurnal Perbenihan Tanaman Hutan, 7(*1), 21-30.

Bareke, T. (2018). Biology of seed development and germination physiology. *Advances in Plants & Agriculture Research*, *8*(4), 336-346.

Baskin, J. M., & Baskin, C. C. (2004). A classification system for seed dormancy. *Seed science research*, *14*(1), 1-16.

Bewley, J. D., & Black, M. (1994). Seeds: physiology of development and germination.

Bonner, F. T. (1996). Responses to Drying of Recalcitrant Seeds of Quercus nigraL. *Annals of Botany*, *78*(2), 181-187.

Bradford, K. J. (1994). Water stress and the water relations of seed development: a critical review. *Crop Science, 34(*1), 1-11.

Clark, J. R., Stafne, E. T., Hall, H. K., & Finn, C. E. (2007). Blackberry breeding and genetics. *Plant breeding reviews, 29*, 19.

Copeland, L. O., McDonald, M. B., (2001). Seed vigor and vigor testing. Principles of seed science and technology, 165-191.

Deepika, Ankit, Sagar, S., & Singh, A. (2020). Dark-induced hormonal regulation of plant growth and development. *Frontiers in Plant Science*, *11*, 581666.

De Mendiburu, F. (2020). Agricolae: Statistical Procedures for Agricultural Research. https://CRAN.R-project.org/package=agricolae

De Wit, M., Galvão, V. C., & Fankhauser, C. (2016). Light-mediated hormonal regulation of plant growth and development. *Annual review of plant biology*, *67*(1), 513-537.

Fuentes, L., Figueroa, C. R., & Valdenegro, M. (2019). Recent advances in hormonal regulation and cross-talk during non-climacteric fruit development and ripening. *Horticulturae, 5(*2), 45.

Fredes, M., Muñoz, C., Prat, L., Torres, F., Sáez, P., Bustamante, L., ... & Pertuzé, R. (2016). Seed morphology and anatomy of Rubus geoides Sm. *Chilean journal of agricultural research*, *76*(3), 385-389.

Gansberger, M., Stüger, H. P., Weinhappel, M., Moder, K., Liebhard, P., von Gehren, P., ... & Ratzenböck, A. (2017). Germination characteristic of Silphium perfoliatum L. seeds. *Die Bodenkultur*, *68*(2), 73-79.

Gundala, B. T., Widajati, E., & Ilyas, S. (2023). The Development of Germination Tests and Breaking Dormancy Techniques of Bitter Seeds (*Andrographis paniculata* (Burm. f.) Wall. ex Nees). *Journal of Tropical Crop Science, 10(*02), 124–132.

Gupta, R., & Chakrabarty, S. K. (2013). Gibberellic acid in plant: still a mystery unresolved. *Plant signaling & behavior*, *8*(9), e25504.

Ilyas, S. (2012). *Ilmu dan teknologi benih: teori dan hasil-hasil penelitian*. PT Penerbit IPB Press.

International Seed Testing Association (ISTA) (2018). International Rules for Seed Testing, Basserdorf, Switzerland.

Kartika E., Ilyas S., 1994. Pengaruh tingkat kemasakan benih dan metode konservasi terhadap vigor benih dan vigor kacang jogo (*Phaseolus vulgaris* L.). *Buletin Agronomi 22*, 44-59.

Kartika, E., & Ilyas, S. (1994). Effect of seed maturity levels and conservation methods on seed and seedling vigor of broad bean (*Phaseolus vulgaris L*.). *Buletin Agronomi (Indonesia), 22(*2), 44-59.

Kołodziejek, J., Patykowski, J., & Wala, M. (2017). Effect of light, gibberellic acid and nitrogen source on germination of eight taxa from dissapearing European temperate forest, Potentillo albae-Quercetum. *Scientific Reports*, *7*(1), 13924.

Marbach, I., & Mayer, A. M. (1974). Permeability of seed coats to water as related to drying conditions and metabolism of phenolics. *Plant physiology*, *54*(6), 817-820.

Marcos-Filho, J. (2016). Seed physiology of cultivated plant. ABRATES, Londrina, Brazil.

NASA National Aeronautics and Space Administration, NASA Power Data Acces Viewer [online], available at https://power.larc.nasa.gov/data-access-viewer/

Normasiwi, S., Salamah, A., & Surya, M. I. (2021). Morphological characteristics of Indonesian Rubus flowers. *Biodiversitas: Journal of Biological Diversity, 22(*3), 1441-1447.

Perkins-Veazie, P., & Nonnecke, G. (1992). Physiological changes during ripening of raspberry fruit. *HortScience*, *27*(4), 331-333.

Rori, H. F., Rampe, H. L., & Rumondor, M. Uji Viabilitas dan Vigor Biji Sirsak (*Annona Muricata L.*) Setelah Aplikasi Kalium Nitrat (Kno3). Jurnal Ilmiah Sains, 18(2), 80-84.

Rori, H. F., Rampe, H. L., & Rumondor, M. (2018). Uji viabilitas dan vigor biji sirsak (*Annona muricata L.*) setelah aplikasi kalium nitrat (KNO3). *Jurnal Ilmiah Sains*, 80-84.

Royal Horticultural Society (RHS), 2015. RHS Colour Chart sixth edition. Royal Horticultural Society, London.

Rusmin, D., & Darwati, I. (2018). Studi Fenologi Dan Penentuan Masak Fisiologis Benih Purwoceng Phenological Study and Determination of Physiological Maturity of *Purwoceng* Seeds. *Jurnal agronida* 1,1, 45-54.

Sadjad, S. (1994). Kuantifikasi metabolisme benih. *PT. Gramedia Widiasarana Indonesia, Jakarta*, *145*.

Sadjad, S., Murniati, E., & Ilyas, S. (1999). Parameter pengujian vigor benih dari komparatif ke simulatif. *Grasindo. Jakarta*, *185*.

Santos, T. P., Sá, M. E., Malagutti, E. S., Pinto, M. S., Ferreira, A. F. A., Monteiro, L. N. H., ... & Rodrigues, M. G. F. (2022). Effects of gibberellic acid concentration and fruit maturation stage on seed germination and vigor of *pitahaya* seedlings. *Brazilian Journal of Biology*, *84*, e260650.

Suharsi, T. K., Syukur, M., & Wijaya, A. R. (2015). Karakterisasi buah dan penentuan saat masak fisiologi benih beberapa genotipe cabai (Capsicum annuum L.). *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, *43*(3), 207-212.

Surya, M. I., Suhartati, S., Ismaini, L., Lusini, Y., Destri, D., Anggraeni, D., Normasiwi S., Asni N., & Sidiq, M. A. B. (2018). Fruit Nutrients of Five Species of Wild Raspberries (*Rubus spp*.) from Indonesian Mountainâ€™ s Forests. *Journal of Tropical Life Science, 8(*1), 75-80.

Wada, S., & Reed, B. M. (2011a). Optimized scarification protocols improve germination of diverse Rubus germplasm. *Scientia Horticulturae, 130(*3), 660-664.

Wada, S., & Reed, B. M. (2011b). Standardizing germination protocols for diverse raspberry and blackberry species. *Scientia Horticulturae, 132*, 42-49.

Wada, S., Kennedy, J. A., & Reed, B. M. (2011). Seed-coat anatomy and proanthocyanidins contribute to the dormancy of Rubus seed. *Scientia Horticulturae*, *130*(4), 762-768.

Warpeha, K. M., & Montgomery, B. L. (2016). Light and hormone interactions in the seed-to-seedling transition. *Environmental and Experimental Botany*, *121*, 56-65.

Włodarczyk, Z., Ziernicka-Wojtaszek, A., Kędzior, R., & Mazur, J. (2023). Flowering phenology of shrub roses as a sensitive indicator of meteorological variability in Central Europe. *Journal of Horticultural Research*, *31*(2), 175-190.

Żurawicz, E., Masny, A., Kubik, J., & Lewandowski, M. (2017). Germination of red raspberry seeds as affected by origin and chemical scarification. *Horticultural Science, 44(*3), 133-140.

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STRESS RESPONSES AND EVALUATION OF PHYTOREMEDIATION POTENTIAL IN ORNAMENTAL KALE SEEDLINGS EXPOSED TO NICKEL

Nickel Stress and Phytoremediation Potential in Ornamental Kale Seedlings

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Abstract - Phytoremediation is an environmental friendly and cost-effective approach that uses plants to remove hazardous chemicals from contaminated soil, air, and water. The effects of nickel (Ni) concentration on Kamome Red, Kamome White and Kamome Pink cultivars of ornamental kale were evaluated in this study. For this purpose, seeds of cultivars were grown in perlite and were irrigated with 0 (control), 25, 50, 100 ppm Ni solution including Hoagland nutrient solution for 40 days. Results showed that the germination percentage was more than 70% in all cultivars and applications. At a Ni concentration of 25 ppm, it had positive effects on seedling growth parameters with 76.1 mm root length, 73.1 mm shoot length, 6.7 g shoot fresh weight, 0.97 g shoot dry weight. However, high Ni concentrations negatively impacted shoot and root lengths, seedling fresh and dry weights. Seedling water content decreased as Ni concentration increased. Proline content increased significantly in response to high Ni concentrations in Kamome Red and Kamome White cultivars. The Ni concentrations of the cultivars were also affected, with the highest Ni content being obtained from 100 ppm Kamome White (89.32 mg/kg). These findings indicate that Ni concentration has an important effect on the growth of ornamental kale cultivars and K. White showed significant Ni accumulation at 100 ppm. Although Ni uptake occurred, the significant biomass reduction at higher doses limited the phytoremediation potential of the cultivars in the seedling stage. It is recommended to evaluate different Ni concentrations at various growth stages to better assess phytoremediation potential in the future studies.

Keywords: heavy metal, nickel, tolerance index, ornamental kale, phytoremediation

Introduction

Plants are exposed to biotic (bacteria, fungi, viruses) and abiotic (drought, salinity, extreme temperatures, heavy metal pollution) stress factors in natural environments. These stress factors can impact plant growth and yield (Nabi et al., 2019). Heavy metal pollution is a significant abiotic stressor, caused by the release of metallic elements and metalloids from various sources, including industrial waste, secondary metal production, energy production, natural sources like volcanic eruptions and dust or forest fires, fertilizer use, and automobiles (Ghori et al., 2019). Heavy metals are defined as metallic chemical elements and metalloids, with densities greater than 5 g/cm^3, including toxic elements such as chromium (Cr), cobalt (Co), nickel (Ni), cadmium (Cd), and lead (Pb) (Briffa et al., 2020). Ni is a heavy metal with its 8.9 g/cm^3 density that is widely present in the environment (22nd most abundant element) and is indispensable for plants from germination to yield (Sreekanth et al., 2013; Shahzad et al., 2018; Zhang et al., 2020). Plants need Ni between 0.01 and 5.0 µg/g dry weight (Seregin & Kozhevnikova, 2006; Rizwan et al., 2017; Zaid et al., 2019). Nickel participates in several biological functions and enzymes (Shahzad et al., 2018). Plants display symptoms of Ni deficiency when the Ni levels in their leaves are below 1 mg/kg, according to Rue et al. (2020) and Dotaniya et al. (2022).

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However, high concentrations of Ni can have negative impacts on plant anatomy and physiology (Shahzad et al., 2018), such as seed germination, shoot and root growth inhibition (Rahman et al., 2005), iron (Fe) deficiency and chlorosis (Ewais, 1997; Kirkby & Römheld, 2004), photosynthesis and transpiration deterioration (Sheoran et al., 1990; Ahmad et al., 2011). High concentrations of Ni in plant tissues also reduce nutrient uptake and other metabolic processes, cause oxidative damage, alter to membrane stability and change to antioxidant enzyme activities (Borah et al., 2023; Gajewska et al., 2006; Nawaz et al., 2022; Shahzad et al., 2018; Tipu et al., 2021; Yusuf et al., 2011). Nickel contamination is one of the leading sources of heavy metal pollution, coming from industrial discharge, metallurgical and electroplating industries, the use of pesticides, industrial and municipal waste, coal combustion, solid and liquid fuel usage (Borah et al., 2023; Briffa et al., 2020; Chen et al., 2019; Genchi et al., 2020; Ghori et al., 2019; Nabi et al., 2019). Although Ni exist naturally in soil and water, its level is typically lower than 100 mg/kg in soil and 0.005 mg/L in water (Alloway, 2013; Hassan et al., 2019; Shahzad et al., 2018).

Phytoremediation is an eco-friendly and cost-effective approach that uses plants to remove hazardous chemicals from contaminated soil, air, and water (Salt et al., 1998). It exploits the natural ability of some plant species, known as hyperaccumulators, to concentrate pollutants in their tissues (Pilon-Smits, 2005). Phytoremediation has been successfully used worldwide to eliminate contaminants like heavy metals, pesticides, solvents, explosives, and crude oil (Genchi et al., 2020). Phytoremediation employs two main techniques: phytoextraction and phytostabilization (McGrath & Zhao, 2003). Phytoextraction includes removing contaminants with plants from soil, plants absorb and accumulate them in their tissues and are then removed along with harvesting (Pilon-Smits, 2005). Phytostabilization is immobilizing the contaminants through absorption, adsorption in soil, or precipitation in the rhizosphere via plants (Korzeniowska & Stanislawska-Glubiak, 2019).

Phytoremediation potential of plants can be assessed with different parameters, including translocation factor (TF) and tolerance index (TI), as noted by Usman et al. (2019). The TF indicates the ability of the plant to transport heavy metals from the roots to the aerial parts of a plant (Pilon-Smits, 2005). When the TF value is greater than one, it suggests that the plant has an efficient metal transport system, resulting in effective metals translocation from the roots to the aerial parts. However, when the TF value is less than one, it indicates that the plant has an ineffective metal transfer process, leading to metal accumulation in the roots instead of the aerial parts (Korzeniowska & Stanislawska-Glubiak, 2019; Samreen et al., 2021; Yoon et al., 2006).

Ornamental plants can play a role in phytoremediation as they can exhibit characteristics of hyperaccumulation, accumulation, and exclusion of heavy metals (Rocha et al., 2022). It is preferred to use non-edible plant species for phytoremediation purposes. For example, *Cosmos bipinnatus* has been identified as a cadmium hyperaccumulator (Huang et al., 2017), while *Euphorbia milli* has been recognized as a chromium accumulator (Ramana et al., 2015) and *Acacia pycnantha* as a lead excluder (Khan et al., 2021; Nirola et al., 2016). Because it is not preferred to accumulate heavy metals in edible plant species, ornamental kale has potential for phytoremediation as they are not consumed as food or feed. Ornamental kale (*Brassica oleracea* var. *acephala*) is a cultivated ornamental plant that has high commercial value and is widely grown due to its colorful lace-like leaves. It is commonly used as a horticultural decoration due to its biennial growth habit and long-lasting foliage. The leaves of the plant have distinctive shapes and margins that can be lobed, serrated, or entire, with attractive colors (Ari et al., 2021; Chen et al., 2019; Karagöz & Dursun, 2021; Ren et al., 2019). Planting ornamental kale during the autumn and winter months is optimal for growth. Ornamental kale belongs to the Brassicaceae family and is considered one of the oldest vegetable forms, with a long history of cultivation in Europe (Dixon, 2017). Brassica plants can accumulate heavy metals (da Mota Gonçalves et al., 2022; Vannucchi et al., 2021). Research has indicated that ornamental kale can be used as hyperaccumulator plants for Cd, Pb, Zn, and Cu in soils (Haghighi et al., 2016; Nasiri et al., 2022). *Brassica oleracea* var. *acephala*, a subspecies of *Brassica oleracea* and considered an ornamental plant, was found to be a potential hyperaccumulator plant for the improvement of boron-contaminated areas (Gökseven et al., 2021).

The present study had two main objectives: (1) to examine the impact of Ni stress on the germination (germination rate, germination index and mean germination time), growth (weight, length and water content), and physiological parameters (proline and Ni content) of ornamental *Brassica oleracea* seedlings during the vegetative stage; (2) to assess the phytoremediation potential of ornamental kale.

Materials and Methods

Plant material

Ornamental Kale (*Brassica oleracea* L. var. *acephala*) Kamome Pink (K.Pink), Kamome White (K.White), and Kamome Red (K.Red) cultivars were utilized as the plant material in this study. The seeds were sourced from a local seed distributor (Tasaco Farm, Antalya, Turkey) and underwent disinfection with commercial bleach for

1 minute (Taghizadeh et al., 2018) prior to being sown in plastic pots filled with perlite. Each pot was treated with a solution containing one of the four nickel concentrations (0 (Control), 25, 50, and 100 ppm), prepared from $Ni(NO_3)_2$, alongside the standard Hoagland nutrient solution. Each pot contained five seeds, and the experiment was initiated on December 30, 2021, in a controlled climate room with a day/night temperature of 22/15 °C, humidity of 55-65%, photosynthetically active radiation of 300 μmol m^{-2} s^{-1}, and a day/night photoperiod of 14/10 (Pathirana et al., 2017). Plants were irrigated at two-day intervals with 50 ml of the solution. The experiment was conducted at the Suluova Vocational School of Amasya University in Turkey and was terminated on 27 January 2022.

Germination parameters

Seed germination was assessed on the fourth day of the experiment by counting the number of seeds that had germinated. The germination rate, index, and mean germination time were calculated using the following formulas which described by (Çarpıcı et al., 2009) and (Sadeghi et al., 2011):

Germination rate (%) = (Number of germinated seeds / Number of total seeds) *100 (1)

Germination index = ∑(Gt / Tt) (2)

(Gt represents the number of seeds germinated on a given day; Tt is the number of days from the beginning of the experiment until that day.)

Mean germination time (days) = ΣDn / Σn (3)

(n represents the germinated seeds number on day D; D is the days number which counted from first germination)

Seedling growth parameters

Three randomly selected seedlings from each pot were harvested for plant part analysis 40 days after sowing. Shoot and root length were measured with a ruler. The shoot and root sections were separated and their total fresh weight and length (shoot height) were measured.

The water content of the seedlings was calculated using the following formula (Fradera-Soler et al., 2021):

Seedling Water Content (%) = (Seedling fresh weight - seedling dry weight) / Seedling fresh weight *100 (4)

Tolerance index

By subtracting the biomass content of the control plants from that of the treated plants, the tolerance index (TI) can be determined. If the TI values are greater than 1 or less than 1, it indicates an increase or decrease in biomass content, respectively, for the plant cultivars in question. The equation used to calculate TI is provided in Eq. 5, as described by Wilkins (1978) and Borah et al. (2023):

TI (tolerance index) = Biomass of the treated plants (g) / Biomass of the control plants (g) (5)

Proline content

The proline content of the plant material was determined using a modified method of Bates et al. (1973). For this, 100 mg of dried plant material was homogenized with 10 ml of 3% sulfosalicylic acid and filtered through a blue band filter paper. Then, 2 ml of the filtrate was added to a glass tube and mixed with 2 ml of ninhydrin solution (which contains ninhydrin, orthophosphoric acid, and acetic acid) and 2 ml of acetic acid. The mixture was vortexed and then incubated in a 100 °C water bath for one hour, followed by being transferred to an ice bath. Finally, 4 ml of toluene was added to the samples, which were then vortexed and analyzed spectrophotometrically at 520 nm. The proline content was calculated using commercial L-proline as a reference standard.

Nickel concentration

Dried plant shoots (0.2 g) were taken and burned in the muffle furnace at 550 ˚C for 5 hours, 2 ml of HCl (37%) was added and slowly heated on a hot plate. After the acid was removed from the medium 2 ml of 1/3 HCl was added and filtered through a blue band filter paper. The filtrates were made up of 40 ml of distilled water. Prepared samples were analyzed by ICP OES, and the results were given as mg/kg (Miller, 1997; Pequerul et al., 1993).

Translocation factor (TF)

Translocation factor (TF) was determined for 25 ppm Ni application by the following equation (Korzeniowska & Stanislawska-Glubiak, 2019):

TF = metal concentration in shoots (mg/kg) / metal concentration in roots (mg/kg) (6)

Statistical analysis

The study was conducted using a completely randomized design with two factors (cultivar and Ni concentration). The treatments had four replications with five plants per replication. The homogeneity of the data was verified using the Levene’s test. The quantitative data, expressed as percentages, were transformed using the arcsine transformation. To analyze the data, two-way Analysis of Variance (ANOVA) was used, and the Least Significant Difference (LSD) multiple range test at a significance level of p≤0.05 was used to compare the means. The coefficient of variation (CV) was determined for determining reliability. Statistical analyses were performed using JMP Software (Version 14.3, SAS Institute Inc., Cary, NC, 1989-2021). Correlation analysis with heatmap and principal component analysis were performed with Python 3.11.0. GraphPad-Prism 8.3.0 was used to design graphics.

Results

Germination parameters

The interaction between Ni and cultivar significantly affected the germination parameters, as shown by statistical analysis (Table 1). Overall, the germination percentage was found to be up to 70%. The highest germination percentage was found in the interactions of 0 ppm Ni + K.Pink, 25 ppm Ni + K.Red, and 0 ppm Ni + K.White. The K.Pink cultivar had the longest germination time in the 50 ppm Ni application. On the other hand, Ni shortened the germination time in K.Red and K.White. The germination index was lowest in K.Pink +50 ppm (2.96) and K.Red + 0 ppm (3.63).

Plant Growth Parameters

In the present study, the impact of cultivars on ornamental kale's shoot length and seedling dry weights was significant, statistically, but not for root length and seedling fresh weight (as shown in Table 2).
The shoot length was the highest in the K.Red cultivar (49.8 mm). On the other hand, the seedling dry weight was the lowest in the K.Pink cultivar (0.35 g).
The effect of Ni stress on the Ni concentrations, shoot and root length, seedling fresh and dry weights was statistically significant (Fig. 1).
Shoot length (Fig. 1A), root length (Fig. 1B), seedling fresh weight (Fig. 1C), and seedling dry weight (Fig. 1D) increased under 25 ppm Ni treatment. These seedling growth parameters dramatically decreased under 50 and 100 ppm Ni treatments.
The results of the study revealed that the interaction between cultivar and Ni concentration had a significant impact on the seedling growth parameters of Kamome cultivars (K. Pink, K.Red, and K.White), with the exception of shoot fresh weight (Table 3).
The longest shoot length was found in the 0 ppm Ni and K.Red cultivar (84.2 mm). A 25 ppm concentration of Ni resulted in an increase in the shoot length of both K.Pink (76.3 mm) and K.White (67.0 mm). The highest root length (90.4 mm) was found in K. Red and 25 ppm interaction. In all cultivars, 25 ppm induced the root length under 25 ppm Ni. As Ni content increased root length decreased in all cultivars. Seedling dry weight was higher in K.White cultivar under 25 ppm Ni (1.11 g).

Seedling water, proline and nickel Content

The results indicated a significant difference ($p < 0.05$) between cultivars in terms of seedling water,, proline and nickel content was significant (Table 4).
Seedling water (79%) and proline content (0.245) were lower in the K.White cultivar. Proline content was the highest in K.Pink (0.245). Additionally, Ni content was highest in K.White (30.69), followed by K.Pink (18.38) and K.Red (15.11), respectively.
The effect of Ni stress on the seedling water, proline and Ni content was important and statistically significant (Fig. 2).
Seedling water content decreased with increasing Ni concentration (Fig. 2A). On the other hand, the proline content increased in response to Ni treatment (Fig. 2B). Results showed that there was slightly Ni uptake with 25 ppm Ni treatment (1.44 mg/kg). When the Ni concentration was 50 and 100 ppm, the Ni concentrations in the seedling also increased, with the highest Ni concentrations observed in the 100 ppm Ni treatment (60.20 mg/kg) (Fig. 2C).
When considering cultivar and Ni interactions, the highest seedling water content was recorded for the combination of 0 ppm Ni and K.Red cultivar (91%) (Table 5).
The lowest water content was noted for 100 ppm Ni and K.White cultivar (69%). The highest proline value was found in the combination of 100 ppm Ni and K.Red cultivar (0.37 mM), while the lowest value was observed in both 0 ppm Ni and K.White cultivar and K.Red cultivar (0.02 mM). The increasing percentage of proline was high in K.Red and K.White under Ni treatments, but it was low in K.Pink.
Roots and shoots of the Kamome cultivars couldn't grow well under 50 and 100 ppm Ni treatments, at least, we could not have enough samples separately as root and shoot, to perform Ni analysis. For this reason, plantlets could not divided into roots and shoots in these concentrations. The Ni concentration of the roots and shoots belongs to Kamome cultivars under 0 and 25 ppm was shown in Fig. 3A. The K.Red and K.White accumulated low amount of Ni in the roots but not in the shoots. When considering all Ni concentrations for all Kamome cultivars, the impact of Ni treatments on the Ni content of the cultivars was found to be statistically significant (Fig. 3B). The Ni content increased with Ni applications, with the highest Ni concentrations found in the 100 ppm K.White cultivar (89.32 mg/kg).

Table 1. Effects of Ni and cultivar interaction on germination parameters of K.Pink, K.Red and K.White.

Kamome Cultivars	Ni (ppm)	Germination (%)	Germination time	Germination index
K.Pink	0	100 (90)±0a	2.66±0.1cde	8.33±1.0ab
	25	95 (83) ±10ab	2.77±0.1c	7.02±1.3abc
	50	70 (57) ±12c	3.19±0.2a	2.96±0.7d
	100	95 (83) ±10ab	2.69±0.2cde	7.81±2.0abc
K.Red	0	70 (57) ±11.5c	2.95±0.1b	3.63±1.1d
	25	100 (90) ±0a	2.75±0.1cd	7.54±0.6abc
	50	95 (83) ±10ab	2.79±0.1bc	6.40±1.1bc
	100	75 (60) ±10c	2.73±0.2cde	5.94±1.7c
K.White	0	100 (90) ±0a	2.71±0.2cde	8.17±2.1abc
	25	95 (83) ±10ab	2.57±0.1de	9.27±2.3a
	50	80 (70) ±23bc	2.55±0.1e	7.83±2.5abc
	100	95 (83) ±10ab	2.56±0.1e	9.06±1.4a
LSD		*16.266****	*0.182****	*2.303***
CV (%)		*12.1*	*4.6*	*22*

**p<0.01, ***p<0.001, CV: coefficient of variation.

Values in parenthesis were transformed with arcsine. Measurements are presented as mean ± standard deviation. The differences between the averages were indicated by separate letters.

Table 2. Effects of cultivars on seedling growth parameters.

Cultivars	Shoot Length (mm)	Root length (mm)	Seedling Fresh Weight (g)	Seedling Dry Weight (g)
K.Pink	41,6b	36,7	2,81	0,35b
K.Red	49,8a	41,5	2,91	0,44a
K.White	41,0b	37,6	3,17	0,49a
LSD	*2.527****	---NS	---NS	*0.049****
CV (%)	*10*	*20*	*23*	*13,9*

***p<0.001, ---NS: nonsignificant, CV: coefficient of variation, Measurements are presented as mean. The differences between the averages were indicated by separate letters.

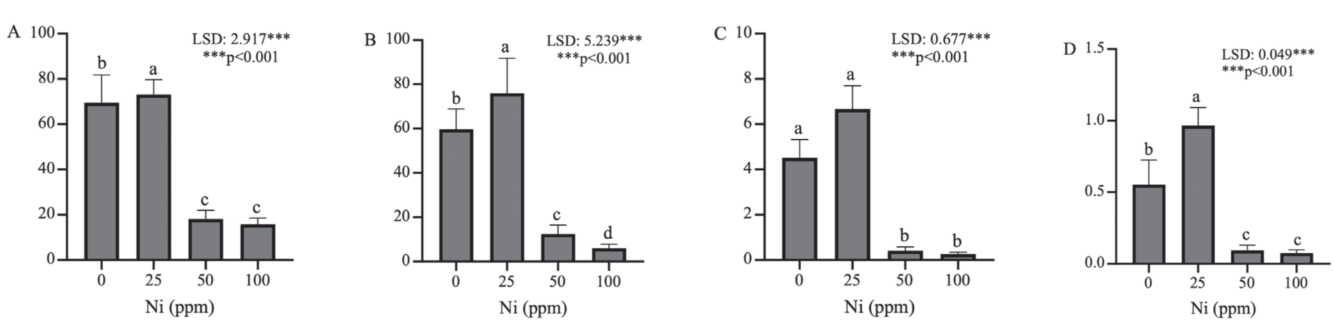

Figure 1. Effects of Ni concentrations on seedling growth parameters. *The differences between the averages were indicated by separate letters.*

Tolerance Index and Translocation Factor

Tolerance index and translocation factor were evaluated by heatmap graphics. Tolerance index was higher in 25 ppm Ni than 50 and 100 ppm Ni. Tolerance index was >1 in 25 ppm Ni in three cultivars and <1 under 50 and 100 ppm Ni (Fig. 4A). While translocation factor of K.White was calculated as 0.00048, it was 0.00045 for K.Red (Fig. 4B). Translocation factor was less than 1 in these cultivars, meanly they could not transfer the Ni from roots to the shoots under 25 ppm Ni concentration.

Heatmap correlation and principal component analysis

Correlation coefficients-heatmap analysis of germination parameters, seedling growth parameters, Ni and proline results in response to different cultivar and Ni applications are shown in Fig. 5A. Positive correlation was determined between the germination percentage and germination index. Germination time had negative correlation with them. Additionally, the seedling water content was positively correlated with the seedling growth parameters and tolerance index. On the other hand, there were significant negative correlations between seedling water content and Ni and proline content. There was not significant correlation between Ni content and the germination parameters. Ni and the seedling growth parameters had negative correlation, while Ni and proline content had positive correlation. Positive and negative correlations between parameters was also showed with the principal component analysis (Fig. 5B).

Discussion

Nickel is a vital microelement for growth and is essential in enzyme synthesis in plants. Ni is beneficial to plants at low concentrations, but high concentrations can be toxic (Das et al., 1978; Hassan et al., 2019). Ni toxicity can reduce the metabolic processes, cell division, cell wall elasticity, and protein synthesis. It can also decrease the activities of essential enzymes such as α-amylase, protease, and nuclease which are important for germination and growth (Ashraf et al., 2011; Bishnoi et al., 1993; Hassan et al., 2019; Seregin & Kozhevnikova, 2006; Sethy & Ghosh, 2013; Walker et al.,1985). In this study, Ni treatment decreased germination time in K.Red and K.White, while germination percentage increased with low Ni levels in K.Red. K.White's germination percentage slightly decreased, but Ni extended the germination time and reduced the germination in K.Pink. Lower level Ni promoted germination rate and seedling growth but high levels inhibited them in rice (Das et al., 1978). Sunflower cultivars showed a significant decrease in their germination rates with the addition of inorganic Ni, except for control seedlings that had the highest germination rate (Zhang et al., 2020). Increasing concentrations of Ni also decreased the germination percentage and rate in rice and lentil seeds (Khan et al., 2020; Shaukat et al., 2021). Barley seeds have also been negatively affected by Ni toxicity (Vasić et al., 2020).

The impact of Ni on plant growth varies depending on the species and level of soil contamination. Korzeniowska & Stanislawska-Glubiak, (2019) found that Ni reduced the

Table 3. Effects of Ni and cultivar interaction on seedling growth parameters.

Cultivars	Ni (ppm)	SL (mm)	RL (mm)	SFW (g)	SDW (g)
K.Pink	0	63.0±7.3cd	56.2±8.8d	4,03±0.84	0,35±0.12d
	25	76.3±6.1b	72.2±7.4b	6,55±0.47	0,85±0.09b
	50	13.3±2.6g	13.3±6.8e	0,31±0.18	0,09±0.05e
	100	13.6±2.2g	5.0±1.2e	0,34±0.04	0,10±0.01e
K.Red	0	84.2±4.9a	58.0±9.8cd	4,42±0.86	0,63±0.05c
	25	76.0±6.2b	90.4±16.9a	6,42±0.80	0,95±0.06b
	50	20.8±1.3e	11.5±2.3e	0,55±0.06	0,11±0.01e
	100	18.3±2.6efg	6.0±1.3e	0,25±0.07	0,07±0.02e
K.White	0	61.5±6.9d	65.2±8.5bcd	5,07±0.60	0,68±0.08c
	25	67.0±2.4c	65.7±10.2bc	7,03±1.75	1,11±0.02a
	50	20.2±0.9ef	12.5±0.8e	0,38±0.19	0,09±0.04e
	100	15.3±0.8fg	7.0±2.4e	0,19±0.04	0,06±0.01e
LSD		*5.053****	*9.076****	---^{NS}	*0.099****

****p<0.001* ---^{NS}*: nonsignificant,* Measurements are presented as mean ± standard deviation. The differences between the averages were indicated by separate letters.

Table 4. Effects of cultivars on seedling water content (SWC), proline and Ni content.

Cultivars	SWC (%)	Proline (mM)	Ni (mg/kg)
K.Pink	81a	0,245a	18,38b
K.Red	81a	0,220b	15,11c
K.White	79b	0,166c	30,69a
LSD	*1.696***	*0.014****	*0.272****
CV (%)	*2,5*	*7,7*	*1,5*

***p<0.01, ***p<0.001,* CV: coefficient of variation. Measurements are presented as mean. The differences between the averages were indicated by separate letters.

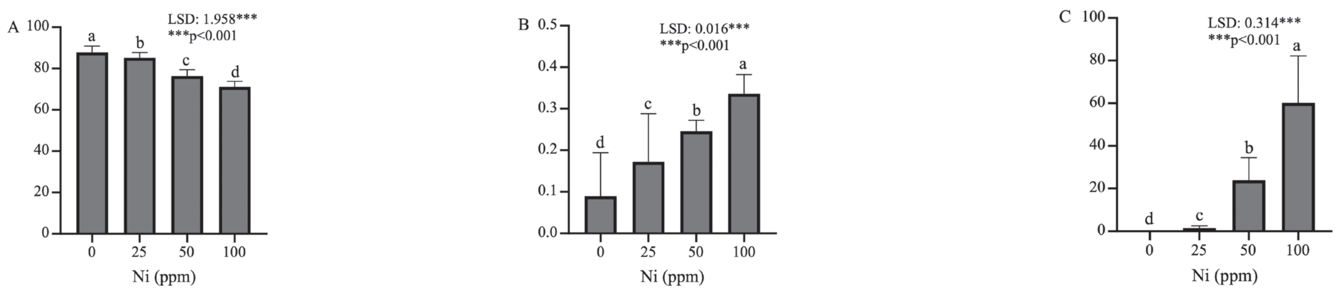

Figure 2. Effects of Ni concentrations on Seedling Water Content (A), proline content (B) and Ni content (C). *The differences between the averages were indicated by separate letters.*

biomass of both aboveground and root parts in plants. In our study, we found that 25 ppm Ni stimulated seedling growth, while 50 and 100 ppm Ni inhibited growth drastically. However, Ni at lower concentrations can stimulate growth but inhibit it at higher concentrations, as observed in rice (Das et al., 1978). Additionally, Ni contamination in soil can reduce both aboveground and root growth, except for *S. viminalis* (Korzeniowska & Stanislawska-Glubiak, 2019). The effect of Ni on sunflower cultivars showed that it had a pronounced effect on seedling shoot and root fresh and dry weights and the highest weights were recorded in the control group followed by lower concentrations of Ni (Ashraf et al., 2011). Ni stress can reduce vital processes like the breakdown of starch into sugars and protein synthesis required for germinating seeds to initiate growth and development (Ashraf et al., 2011). Excessive amounts of Ni is negatively affect the plant growth, photosynthesis, and membrane functions (Maheshwari & Dubey, 2007; Sresty & Rao, 2000). Sresty & Rao (2000) found that two pigeonpea cultivars (*Cajanus cajan* (L.) Millspaugh) were studied for the accumulation of Zn and Ni in their seedlings under different concentrations. Both Zn and Ni applications reduced the above and underground parts growth, as well as the relative growth indices of the two cultivars. Plant parts lengths and biomass of rice seedlings (*Oryza sativa* L. cv. Pant-12) reduced, due to Ni (200 and 400 μM $NiSO_4$). The Ni level was higher in the roots compared to aerial parts (Maheshwari & Dubey, 2009). Studies have also reported negative effects of boron (another heavy metal) on kale growth, specifically in root-shoot fresh and dry weights (Gökseven et al., 2021). Similar results were seen in the case of *Calendula tripterocarpa*, where high Ni concentrations reduced plant growth, including germination and fresh and

Table 5. Effects of Ni and cultivar interaction on seedling water content (SWC) and proline.

Cultivars	Ni (ppm)	SWC (%)	Proline (mM)
K.Pink	0	91±1.5a	0,23±0.001c
	25	87±2.12b	0,24±0.006c
	50	75±3.10d	0,23±0.003c
	100	70±1.06e	0,28±0.003b
K.Red	0	86±1.87b	0,02±0.001d
	25	85±0.92b	0,26±0.025bc
	50	79±1.18c	0,24±0.034c
	100	74±0.64d	0,37±0.007a
K.White	0	87±0.93b	0,02±0.001d
	25	84±3.45b	0,02±0.003d
	50	75±2.59d	0,27±0.002b
	100	69±2.37e	0,36±0.036a
LSD		*1.958****	*0.027****

****p<0.001* Measurements are presented as mean ± standard deviation. The differences between the averages were indicated by separate letters.

dry weights (Heidari et al., 2020). According to Sreekanth et al., (2013) and Valivand et al., (2019), root cell membrane structure changed, water and mineral uptake reduced, and physiological activity affected negatively with high Ni doses. Lešková et al., (2020) also reported that high Ni concentrations reduced auxin transport in roots, damaged the composition of microtubules, affected cell division and, in turn, decreased root growth. Various parameters, including the bioaccumulation factor (BAF), translocation factor (TF), tolerance index (TI), and metal removal efficiency (%), can be used to assess the phytoremediation potential of the plants (Borah et al., 2023; Samreen et al., 2021). Tolerance index was >1 in K.Pink (1.62), K.Red (1.45) and K.White (1.39) under 25 ppm Ni treatment. When Ni concentration were 50 and 100 ppm, TI decreased. Borah et al., (2023) reported that, to assess phyto-tolerance, TI was calculated for *Jatropha curcas* and *Pongamia pinnata* in different soil combinations. Pongamia in PMT25 (municipal landfill 25% : control soil 75%) showed the highest TI value (1.67), indicating greater tolerance than other treatments. Jatropha had TI values above 1 (1.02, 7.55, and 1.09 in PT50 [papermill contaminated soil 50% : control soil 50%], PT25 [papermill contaminated soil 25% : control soil 75%], and MT50 (municipal landfill 50%: control soil 50%), respectively), suggesting a robust defense system, which are heavy metal stress tolerance mechanisms. They supposed that lower TI values at high contaminated

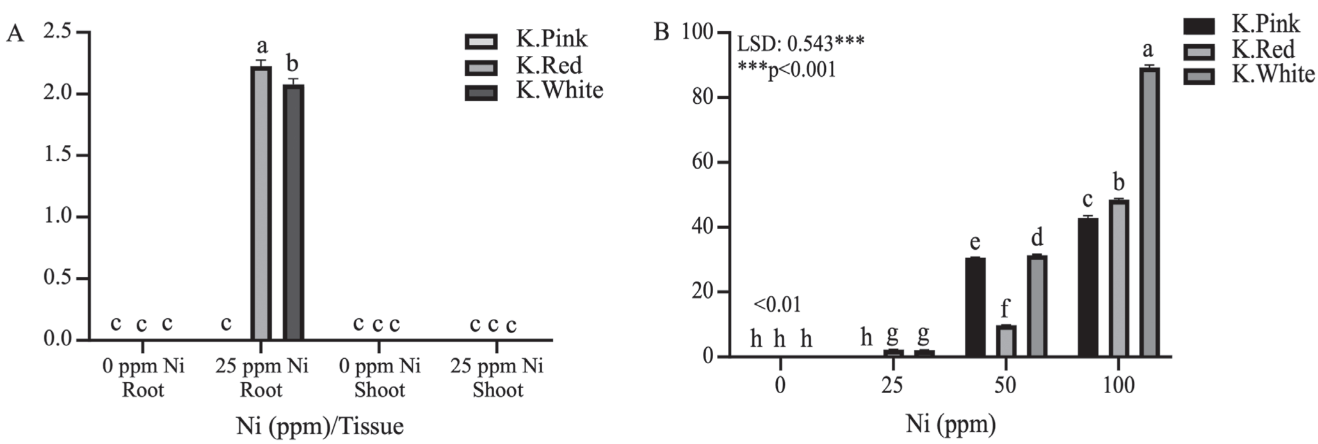

Figure 3. A) Root-Shoot Ni content of three different cultivars under control and 25 ppm Ni B) Effects of Ni and cultivar interaction on Ni content. *The differences between the averages were indicated by separate letters.*

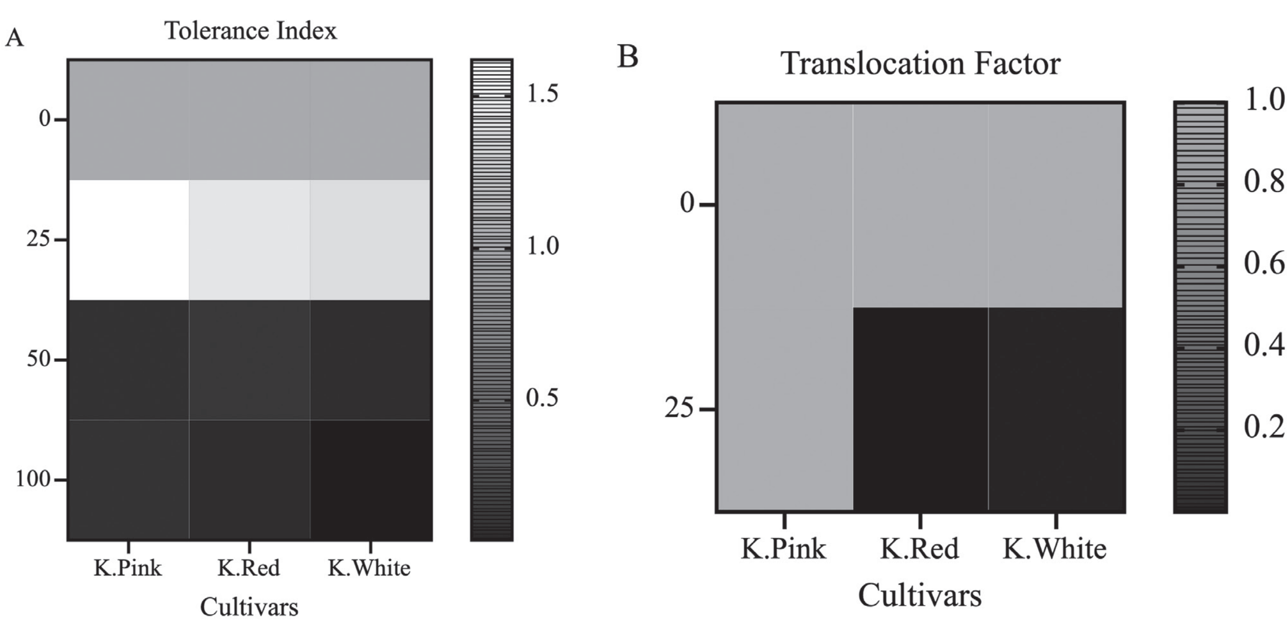

Figure 4. A) Tolerance index of the cultivars under different Ni concentrations, B) Translocation factor of cultivars under control and 25 ppm Ni.

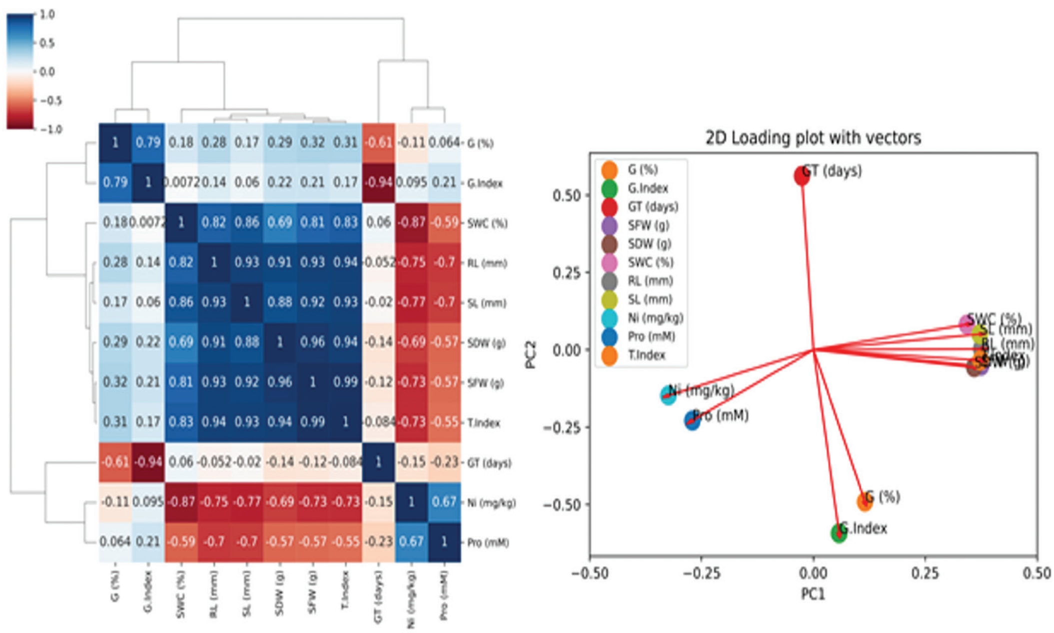

Figure 5. Heatmap showing the levels of on seedling vegetative parameters, proline and Ni content G (%): germination, G.Index: Germination Index, GT (days): Germination time, SFW (g): Shoot fresh weight, SDW (g): Shoot dry weight, SWC (%): Seedling water content, RL (mm): Root Length, SL: Shoot Length, Ni (mg/kg): Ni content, Pro (mM): proline.

area shows lower Ni and Zn tolerance.

The level of proline accumulation in plants indicates of their heavy metals tolerance. Heavy metals caused to increase the content of proline importantly than the control, indicating their metal tolerance capacity in *Jatropha curcas* and *Pongamia pinnata* (Borah et al., 2023). Proline, a common metabolic compound, was found in plant tissues in response to stress conditions (Gajewska et al., 2006). The K.Red and K.White cultivars showed an important increase in proline content under high Ni concentrations, as indicated in Table 3. Proline was described as a protective compound against various stress, with some properties such as osmoprotectant, a membrane stabilizer, and a scavenger of reactive oxygen species (Bandurska, 2001; Hartzendorf & Rolletschek, 2001; Matysik et al., 2002; Gajewska et al., 2006). Schat et al. (1997) emphasized that metal-induced proline production was a response of metal-induced water deficit in the *Silene vulgaris* leaf tissues. Similarly, in our study the decrease in water content of kale seedlings and proline accumulation under Ni presence, may reflect this response. Heavy metals can disrupt water transport in plants, leading to shoot dehydration. Transition metals can affect water uptake, movement, and stomatal function at multiple stages of the process (Yusuf et al., 2011).

Ni moves from roots to shoots and leaves via xylem's transpiration stream, facilitated by cation ATPases, ion channels, and cation-proton antiport. In addition, Ni is moved from older leaves, buds, fruits, and seeds to meristematic parts through the phloem, which is regulated by metal-ligand complexes and specific proteins that bind Ni (Colpas & Hausinger, 2000; Yang et al., 2005). In our own study, we found that the Ni content in K.Pink, K.Red, and K.White ornamental kale seedlings increased with high Ni content, with K.Red and K.White accumulating the most in their roots when exposed to 25 ppm Ni. Similarly, the roots of *Zea mays* contained significantly more Ni compared to shoots, with the plant showing a high ability to accumulate Ni in the roots, as found by Korzeniowska and Stanislawska-Glubiak (2019). In maize, lettuce and field bean plants also showed a much higher Ni accumulation rate in the roots than in the stems as discovered by Antonkiewicz et al. (2016). Additionally, studies have shown that exogenous application of Ni leads to an increase in Ni accumulation in various sunflower tissues (Ahmad et al., 2009; Ashraf et al., 2011). Nonetheless, increasing Ni level in the nutrient solution may have impeded the uptake of essential minerals.

Conclusions

In conclusion, this study evaluated the effect of varying Ni concentrations on the growth parameters of three kale cultivars: K.Pink, K.Red, and K.White. The results indicated that 25 ppm Ni significantly enhanced seedling growth but high Ni levels had a detrimental effect on the growth characteristics of the kale seedlings, including shoot and root length, fresh and dry weight, and water content. Furthermore, the K.Red and K.White cultivars showed a remarkable increase in proline levels in response to high Ni concentrations, suggesting it as a protective mechanism against stress. Additionally, the study observed a statistically significant accumulation of Ni in the seedlings, with the highest concentration found in the 100 ppm K.White cultivar. On the other hand, the 25 ppm K.Pink cultivar exhibited no significant change in Ni content. This study highlights the importance of understanding species-specific responses to nickel stress, which could inform better management practices in cultivating ornamental kale and other plants in contaminated environments.

Author Contributions

Conceptualization: [VC, SY, HOB]; Methodology: [VC, SY, HOB]; Formal analysis and investigation: [VC, SY, HOB]; Writing - original draft preparation: [VC, HOB]; Statistical Analysis and Figure preparation [SY], Writing - review and editing: [VC, SY, HOB].

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest

The authors state no conflict of interest

Funding Declaration

This research received no external funding.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used OPENAI in order to English Grammer Check.

References

Ahmad, M. S. A., Hussain, M., Ashraf, M., Ahmad, R., & Ashraf, M. Y. (2009). Effect of nickel on seed germinability of some elite sunflower (Helianthus annuus L.) cultivars. *Pak J Bot*, *41*(4), 1871-1882.

Ahmad, M. S. A., Ashraf, M., & Hussain, M. (2011). Phytotoxic effects of nickel on yield and concentration of macro-and micro-nutrients in sunflower (Helianthus annuus L.) achenes. *Journal of Hazardous Materials*, *185*(2-3), 1295-1303.

Alloway, B. J. (2013). Heavy Metals and Metalloids as Micronutrients for Plants and Animals. *Heavy Metals in Soils*, 195-209.

Antonkiewicz, J., Jasiewicz, C., Koncewicz-Baran, M., & Sendor, R. (2016). Nickel bioaccumulation by the chosen plant species. *Acta Physiologiae Plantarum*, *38*(2), 40.

Ari, E., Bedir, H., & Mutlu, N. (2021). Enhancement of embryogenesis in freshly isolated microspore cultures of ornamental kale through direct cold shock treatment. *Scientia Horticulturae, 280,* 109961.

Ashraf, M. Y., Sadiq, R., Hussain, M., Ashraf, M., & Ahmad, M. S. A. (2011). Toxic effect of nickel (Ni) on growth and metabolism in germinating seeds of sunflower (Helianthus annuus L.). *Biological trace element research*, *143*(3), 1695-1703.

Bandurska, H. (2001). Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injuries? II. Proline accumulation during hardening and its involvement in reducing membrane injuries in leaves subjected to severe osmotic stress. *Acta physiologiae plantarum*, *23*(4), 483-490.

Bates, L. S., Waldren, R. P. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

Bishnoi, N. R., Sheoran, I. S., & Singh, R. (1993). Effect of cadmium and nickel on mobilisation of food reserves and activities of hydrolytic enzymes in germinating pigeon pea seeds. *Biologia plantarum*, *35*(4), 583-589.

Borah, P., Rene, E. R., Rangan, L., & Mitra, S. (2023). Phytoremediation of nickel and zinc using Jatropha curcas and Pongamia pinnata from the soils contaminated by municipal solid wastes and paper mill wastes. *Environmental Research*, *219*, 115055.

Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon, 6(*9).

Chen, W., Zhang, Y., Ren, J., Ma, Y., Liu, Z., & Hui, F. (2019). Effects of methylene blue on microspore embryogenesis and plant regeneration in ornamental kale (Brassica oleracea var. acephala). *Scientia Horticulturae*, *248*, 1-7.

Colpas, G. J., & Hausinger, R. P. (2000). In Vivo and in Vitro Kinetics of Metal Transfer by the Klebsiella aerogenes Urease Nickel Metallochaperone, UreE* 210. *Journal of Biological Chemistry*, *275*(15), 10731-10737.

Carpıcı, E. B., Celık, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (Zea mays L.) cultivars. *African Journal of Biotechnology*, *8*(19), 4918-4922.

da Mota Gonçalves, R. G., Dos Santos, C. A., da Fonseca Breda, F. A., Lima, E. S. A., do Carmo, M. G. F., de Souza, C. D. C. B., & do Amaral Sobrinho, N. M. B. (2022). Cadmium and lead transfer factors to kale plants (Brassica oleracea var. acephala) grown in mountain agroecosystem and its risk to human health. *Environmental Monitoring and Assessment*, *194*(5), 366.

Das, P. K., Kar, M., & Mishra, D. (1978). Nickel nutrition of plants: I. Effect of nickel on some oxidase activities during rice (Oryza sativa L.) seed germination. *Zeitschrift für Pflanzenphysiologie*, *90*(3), 225-233.

Dixon, G. R. (2017). The origins of edible brassicas. *Plantsman*, *16*(3), 180-185.

Dotaniya, M. L., Pipalde, J. S., Jain, R. C., Selladurai, R., Gupta, S. C., Das Vyas, M., ... & Kumar, A. (2022). Nickel-mediated lead dynamics and their interactive effect on lead partitioning and phytoremediation indices in spinach. *Environmental Monitoring and Assessment*, *194*(5), 334.

Ewais, E. A. (1997). Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. *Biologia plantarum*, *39*(3), 403-410.

Fradera-Soler, M., Rudall, P. J., Prychid, C. J., & Grace, O. M. (2021). Evolutionary success in arid habitats: Morpho-anatomy of succulent leaves of Crassula species from southern Africa. *Journal of Arid Environments*, *185*, 104319.

Gajewska, E., Skłodowska, M., Słaba, M., & Mazur, J. (2006). Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. *Biologia plantarum*, *50*(4), 653-659.

Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human health and environmental toxicology. *International journal of environmental research and public health*, *17*(3), 679.

Ghori, N. H., Ghori, T., Hayat, M. Q., Imadi, S. R., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International journal of environmental science and technology*, *16*(3), 1807-1828.

Gökseven, Ş. B. E., Kıran, S., & Ellialtıoğlu, Ş. Ş. (2022). The effects of humic acid and chelate applications on some morphophysiological properties and antioxidant enzyme activities of ornamental cabbage (Brassica oleracea var. acephala) under boron stress. *Soil Studies*, *11*(2), 85-95.

Haghighi, M., Kafi, M., Pessarakli, M., Sheibanirad, A., & Sharifinia, M. R. (2016). Using kale (Brassica oleracea var. acephala) as a phytoremediation plant species for lead (Pb) and cadmium (Cd) removal in saline soils. *Journal of Plant Nutrition*, *39*(10), 1460-1471.

Hartzendorf, T., & Rolletschek, H. (2001). Effects of NaCl-salinity on amino acid and carbohydrate contents of Phragmites australis. *Aquatic Botany*, *69*(2-4), 195-208.

Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Aamer, M., Nawaz, M., ... & Khan, T. A. (2019). Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. *Environmental Science and Pollution Research*, *26*(13), 12673-12688.

Heidari, J., Amooaghaie, R., & Kiani, S. (2020). Impact of chitosan on nickel bioavailability in soil, the accumulation and tolerance of nickel in Calendula tripterocarpa. *International Journal of Phytoremediation*, *22*(11), 1175-1184.

Huang, J., Yang, Z., Li, J., Liao, M. A., Lin, L., Wang, J., ... & Ren, W. (2017). Cadmium accumulation characteristics of floricultural plant Cosmos bipinnata. *Chemistry and Ecology*, *33*(9), 807-816.

Karagöz, F. P., & Dursun, A. (2021). Calcium nitrate on growth and ornamental traits at salt-stressed condition in ornamental kale (Brassica oleracea L. var. Acephala). *Ornamental horticulture*, *27*(2), 196-203.

Khan, A. H. A., Kiyani, A., Mirza, C. R., Butt, T. A., Barros, R., Ali, B., ... & Yousaf, S. (2021). Ornamental plants for the phytoremediation of heavy metals: Present knowledge and future perspectives. *Environmental Research*, *195*, 110780.

Khan, M. A., Saeed, S., Ullah, N., Rukh, S., Javed, M. S., Amjad, A., ... & Shah, M. (2020). Effect of nickel on the germination and biochemical parameters of two rice varieties. *Fresenius Environmental Bulletin*, *29*(2), 956-963.

Kirkby, E. A., & Römheld, V. (2004). Micronutrients in plant physiology: functions, uptake and mobility. *Proceedings - International Fertiliser Society, 543,* 1-52.

Korzeniowska, J., & Stanislawska-Glubiak, E. (2019).

Phytoremediation potential of Phalaris arundinacea, Salix viminalis and Zea mays for nickel-contaminated soils. *International Journal of Environmental Science and Technology*, *16*(4), 1999-2008.

Lešková, A., Zvarík, M., Araya, T., & Giehl, R. F. (2020). Nickel toxicity targets cell wall-related processes and PIN2-mediated auxin transport to inhibit root elongation and gravitropic responses in Arabidopsis. *Plant and Cell Physiology*, *61*(3), 519-535.

Maheshwari, R., & Dubey, R. S. (2007). Nickel toxicity inhibits ribonuclease and protease activities in rice seedlings: protective effects of proline. *Plant Growth Regulation*, *51*(3), 231-243.

Maheshwari, R., & Dubey, R. S. (2009). Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. *Plant growth regulation*, *59*(1), 37-49.

Matysik, J., Alia, Bhalu, B., & Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Science*, 525-532.

McGrath, S. P., & Zhao, F. J. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current opinion in biotechnology*, *14*(3), 277-282.

Miller, R.O. (1997). High-Temperature Oxidation: Dry Ashing. In: Handbook of reference methods for plant analysis. Kalra, Y. (Ed.). *CRC press*, 53.

Nabi, R. B. S., Tayade, R., Hussain, A., Kulkarni, K. P., Imran, Q. M., Mun, B. G., & Yun, B. W. (2019). Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. *Environmental and Experimental Botany*, *161*, 120-133.

Nasiri, K., Babaeinejad, T., Ghanavati, N., & Mohsenifar, K. (2022). Arbuscular mycorrhizal fungi affecting the growth, nutrient uptake and phytoremediation potential of different plants in a cadmium-polluted soil. *Biometals*, *35*(6), 1243-1253.

Nawaz, H., Ali, A., Saleem, M. H., Ameer, A., Hafeez, A., Alharbi, K., ... & Farid, G. (2022). Comparative effectiveness of EDTA and citric acid assisted phytoremediation of Ni contaminated soil by using canola (Brassica napus). *Brazilian Journal of Biology*, *82*, e261785.

Nirola, R., Megharaj, M., Aryal, R., & Naidu, R. (2016). Screening of metal uptake by plant colonizers growing on abandoned copper mine in Kapunda, South Australia. *International journal of phytoremediation*, *18*(4), 399-405.

Pathirana, I., Thavarajah, P., Siva, N., Wickramasinghe, A. N., & Smith, P. (2017). Moisture deficit effects on kale (Brassica oleracea L. var. acephala) biomass, mineral, and low molecular weight carbohydrate concentrations. *Scientia Horticulturae*, *226*, 216-222.

Pequerul, A., Pérez, C., Madero, P., Val, J., & Monge, E. (1993). A rapid wet digestion method for plant analysis. In Optimization of plant nutrition: refereed papers from the eighth international colloquium for the optimization of plant nutrition, 31 august–8 September 1992, Lisbon, Portugal (pp. 3-6). *Springer* Netherlands.

Pilon-Smits, E. (2005). Phytoremediation. *Annu. Rev. Plant Biol., 56(*1), 15-39.

Rahman, H., Sabreen, S., Alam, S., & Kawai, S. (2005). Effects of nickel on growth and composition of metal micronutrients in barley plants grown in nutrient solution. *Journal of Plant Nutrition, 28*(3), 393-404.

Ramana, S., Biswas, A. K., Singh, A. B., Ajay, Ahirwar, N. K., & Subba Rao, A. (2015). Tolerance of ornamental succulent plant crown of thorns (*Euphorbia milli*) to chromium and its remediation. *International Journal of Phytoremediation, 17(*4), 363-368.

Ren, J., Liu, Z., Du, J., Fu, W., Hou, A., & Feng, H. (2019). Fine-mapping of a gene for the lobed leaf, BoLl, in ornamental kale (Brassica oleracea L. var. acephala). *Molecular breeding, 39(3*), 1-14.

Rizwan, M., Imtiaz, M., Dai, Z., Mehmood, S., Adeel, M., Liu, J., & Tu, S. (2017). Nickel stressed responses of rice in Ni subcellular distribution, antioxidant production, and osmolyte accumulation. *Environmental Science and Pollution Research, 24(*25), 20587-20598.

Rocha, C. S., Rocha, D. C., Kochi, L. Y., Carneiro, D. N. M., Dos Reis, M. V., & Gomes, M. P. (2022). Phytoremediation by ornamental plants: a beautiful and ecological alternative. *Environmental Science and Pollution Research*, *29*(3), 3336-3354.

Rue, M., Paul, A. L., Echevarria, G., van Der Ent, A., Simonnot, M. O., & Morel, J. L. (2020). Uptake, translocation and accumulation of nickel and cobalt in Berkheya coddii, a 'metal crop' from South Africa. *Metallomics*, *12*(8), 1278-1289.

Sadeghi, H., Khazaei, F., Yari, L., & Sheidaei, S. (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean (Glycine max L.). *ARPN Journal of Agricultural and Biological Science*, *6*(1), 39-43.

Salt, D. E., Smith, R. D., & Raskin, I. (1998). Phytoremediation. *Annual review of plant biology*, *49*(1), 643-668.

Samreen, S., Khan, A. A., Khan, M. R., Ansari, S. A., &

Khan, A. (2021). Assessment of phytoremediation potential of seven weed plants growing in chromium-and nickel-contaminated soil. *Water, Air, & Soil Pollution*, *232*(5), 209.

Schat, H., Sharma, S. S., & Vooijs, R. (1997). Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of Silene vulgaris. *Physiologia plantarum*, *101*(3), 477-482.

Seregin, I., & Kozhevnikova, A. D. (2006). Physiological role of nickel and its toxic effects on higher plants. *Russian journal of plant Physiology*, *53*(2), 257-277.

Sethy, S. K., & Ghosh, S. (2013). Effect of heavy metals on germination of seeds. *Journal of natural science, biology, and medicine*, *4*(2), 272.

Shahzad, B., Tanveer, M., Rehman, A., Cheema, S. A., Fahad, S., Rehman, S., & Sharma, A. (2018). Nickel; whether toxic or essential for plants and environment-A review. *Plant physiology and biochemistry*, *132*, 641-651.

Shaukat, S. S., Khan, M. A., & Tayyab, M. S. A. N. (2021). Effect of nickel on germination, seedling growth, chlorophyll content and amylase activity of Lens culinaris L.: an application of appropriate tool for analysis of germination data. *Int J Biol Biotech 18(*3): 509-515.

Sheoran, I. S., Singal, H. R., & Singh, R. (1990). Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeonpea (Cajanus cajan L.). *Photosynthesis Research*, *23*(3), 345-351.

Sreekanth, T. V. M., Nagajyothi, P. C., Lee, K. D., & Prasad, T. N. V. K. V. (2013). Occurrence, physiological responses and toxicity of nickel in plants. *International Journal of Environmental Science and Technology*, *10*(5), 1129-1140.

Sresty, T. V. S., & Rao, K. M. (2000). Organ compartmentation of zinc and nickel in pigeonpea seedlings in relation to cultivar tolerance. *Journal of plant nutrition*, *23*(10), 1421-1434.

Taghizadeh, M., Solgi, M., Karimi, M., Sanati, M. H., & Khoshbin, S. (2018). Heavy metals effects on Brassica oleracea and elements accumulation by salicylic acid. *Archives of Hygiene Sciences*, *7*(1), 1-11.

Tipu, M. I., Ashraf, M. Y., Sarwar, N., Akhtar, M., Shaheen, M. R., Ali, S., & Damalas, C. A. (2021). Growth and physiology of maize (Zea mays L.) in a nickel-contaminated soil and phytoremediation efficiency using EDTA. *Journal of Plant Growth Regulation*, *40*(2), 774-786.

Usman, K., Al-Ghouti, M. A., & Abu-Dieyeh, M. H. (2019). The assessment of cadmium, chromium, copper, and nickel tolerance and bioaccumulation by shrub plant Tetraena qataranse. *Scientific reports*, *9*(1), 5658.

Valivand, M., Amooaghaie, R., & Ahadi, A. (2019). Interplay between hydrogen sulfide and calcium/calmodulin enhances systemic acquired acclimation and antioxidative defense against nickel toxicity in zucchini. *Environmental and Experimental Botany*, *158*, 40-50.

Vannucchi, F., Francini, A., Raffaelli, A., & Sebastiani, L. (2021). Removal of multi-contaminants from water by association of poplar and Brassica plants in a short-term growth chamber experiment. *Environmental Science and Pollution Research*, *28*(13), 16323-16333.

Vasić, P., Jakšić, T., & Đelić, G. (2020). Impact of Pb, Ni and Cd on the germination of barley seeds, variety Jadran. *Bulletin of Natural Sciences Research*, *10*(2), 1-6.

Walker, C. D., Graham, R. D., Madison, J. T., Cary, E. E., & Welch, R. M. (1985). Effects of Ni deficiency on some nitrogen metabolites in cowpeas (Vigna unguiculata L. Walp). *Plant Physiology*, *79*(2), 474-479.

Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist*, *80*(3), 623-633.

Yang, X., Feng, Y., He, Z., & Stoffella, P. J. (2005). Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. *Journal of trace elements in medicine and biology*, *18*(4), 339-353.

Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the total environment*, *368*(2-3), 456-464.

Yusuf, M., Fariduddin, Q., Hayat, S., & Ahmad, A. (2011). Nickel: an overview of uptake, essentiality and toxicity in plants. *Bulletin of environmental contamination and toxicology*, *86*(1), 1-17.

Zaid, A., Mohammad, F., Wani, S. H., & Siddique, K. M. (2019). Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. *Ecotoxicology and environmental safety*, *180*, 575-587.

Zhang, X., Su, C., Liu, X., Liu, Z., Liang, X., Zhang, Y., & Feng, Y. (2020). Effect of plant-growth-promoting rhizobacteria on phytoremediation efficiency of Scirpus triqueter in pyrene-Ni co-contaminated soils. *Chemosphere*, *241*, 125027.

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BOTANICAL AND ARCHAEOLOGICAL HERITAGE IN CUBA: THE CAYO BLANCO-GUASABACOA CASE STUDY BOTANICAL AND ARCHEOLOGICAL HERITAGE IN CUBA

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Abstract - The Archeo-Cuba project, Archaeology and Environmental Sustainability, is an international cooperation initiative funded by the Italian Agency for Development Cooperation under the program for the Promotion of Territorial Partnerships and Implementation of the 2030 Agenda. The project adopts a multidisciplinary and integrated approach to addressing climate change in Cuba, aligning with the goals of the 2030 Agenda, through a peer-to-peer training model involving both Italian and Cuban experts. This paper presents the analytical methods applied to the project's target sites, focusing on the interpretation of natural and botanical stratification alongside historical and archaeological layers. It also outlines key findings from both the botanical research and the planning activities carried out at one of the target sites: the Cayo Blanco area, located in Havana Bay. Furthermore, recognizing the value of botanical, natural, and historical-archaeological heritage as a driver of sustainable territorial development, the study proposes environmental conservation strategies based on Nature-Based Solutions to address both ecological and social challenges. All activities are aimed at the future enhancement and sustainable use of the site.

Keywords: cuba, climate change, environmental sustainability, ethnobotany, nature based solutions

Introduction

Cuba's island, rich in historical and natural heritage, is currently implementing policies strongly oriented toward sustainable development, beginning with urban recovery and regeneration programs. "Archeo-Cuba" is a project aimed at promoting the definition, implementation, and revitalization of territorial public policies in Cuba. These policies focus on identifying, protecting, and enhancing the stratified historical-cultural landscape, alongside the natural environment, moving toward a comprehensive and cross-disciplinary vision of "heritage" in its broadest sense.
The city of Havana, particularly regarding its historic center, had already adopted the "Plan Especial de Desarrollo Integral" (PEDI), a framework comprising essential tools for guiding spatial planning and the integrated development of the historic center. This plan considers culture as a driver of development and places human beings at the center of rehabilitation efforts. It balances the need to valorize and manage cultural and environmental heritage by leveraging the territory's intrinsic resources and renewable energy sources. Moreover, Cuba has recently adopted National Law 155/2023, "Ley General de Protección al Patrimonio Cultural y al Patrimonio Natural", which coordinates the protection of both cultural and natural heritage, explicitly recognizing the interrelationship between these two dimensions.
Additionally, several pre-existing planning instruments in Havana must be considered: the Environmental Strategy for the Urban Conservation Priority Zone (ZPC) of Havana (2013–2020), which outlines priorities for protecting natural

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resources, strengthening climate change mitigation in territorial planning, and applying environmental policy and management tools; the Management Plan for the Cultural Landscape of Havana Bay and surrounding areas; and the state plan Tarea Vida, which addresses climate change impacts through five strategic actions and eleven targeted measures aimed at mitigating the effects of climate change on vulnerable areas.

Due to its geographical position, Cuba is particularly exposed to extreme weather events. According to the most recent studies conducted in 2017 by the Cuban Ministry of Science, Technology, and Environment and the National Meteorological Institute, the climate is changing rapidly. Projections for 2100 suggest an increase of at least 4.5°C in average temperature and a sea-level rise of 85 cm, potentially resulting in the loss of approximately 5.5% of Cuba's land area.

The intervention strategy also draws upon international frameworks and guidelines, such as those of ICOMOS and UNESCO, addressing the connections and potential strategies to mitigate the effects of climate change on cultural heritage. It also aligns with the objectives of the 2030 Agenda for Sustainable Development, particularly SDG 11 (Sustainable Cities and Communities) and SDG 13 (Climate Action) (Ricciardi & Salerno, 2024).

In this context, the project Archeo-Cuba, Archaeology and Environmental Sustainability, was launched with the aim of recognizing the historical and natural values of selected target sites that are strongly representative of Cuba's history and evolution. The project also seeks to highlight the potential negative effects of climate change on these values, while developing mitigation strategies.

Focusing specifically on the botanical component, the project builds on experience gained in Italy in areas of both historical-archaeological and naturalistic interest, such as the Appian Way Regional Park in Rome, the site of "San Vincenzo al Volturno" (Cicinelli et al., 2017), and the site of "Abellinum". In these locations, efforts have been made to identify botanical elements of biogeographical and conservation interest, both for their appropriate long-term management and preservation, and for their cultural valorization. Attention has also been paid to identifying threats, such as invasive alien species. However, the most innovative approach of the Archeo-Cuba project lies in the use of vegetation as a tool for interpreting the "historical stratigraphy" of the sites themselves.

This contribution focuses on one of the five target sites of the Archeo-Cuba project: the area of Cayo Blanco, located in the Bay of Havana with its name derived from the white hue of this characteristic rock. This area displays key characteristics of landscape and historical stratification, and, thanks to a multidisciplinary approach involving scholars and experts, may serve as a pilot site for testing sustainable actions and interventions.

Materials and Methods

Study area

The site under study is located on a small peninsula to the southwest of Havana Bay. An analysis of historical maps (Fig. 1) reveals that this land extension was not always connected to the coastal strip of the bay. It originally appeared as a small island, surrounded by flooded zones, most likely dominated by mangrove ecosystems.

Within this site, at the end of the 18th century, the *Polvorín San Antonio* was constructed (Fig. 2).

This was a colonial-era arsenal comprising a simple rectangular masonry structure, fortified and enclosed by a perimeter wall, which served as a storage facility for weapons and ammunition. It was only in the 1970s, through underwater archaeological investigations, that two indigenous archaeological sites, *Guasabacoa I* and *Guasabacoa II*, were identified nearby (Roura Alvarez, 2025). According to the Management Plan for the Archaeological Heritage of Old Havana, this site is designated as a Priority Area for Conservation and categorized within the Archaeological Risk Zone I granting it the highest level of protection in terms of archaeological significance. Situated in the lower part of Havana Bay, near the mouth of the Luyanó River, the site's exposure to bay currents is particularly pronounced. Over time, sediment accumulation around the former islet has led to its permanent connection to the mainland (Fig. 1). Furthermore, industrial development and urban expansion have resulted in the construction of large platforms supporting shipyard and industrial facilities serving both Havana and the neighboring municipality of Regla to the southeast. These interconnected zones have been infilled with reclaimed material.

Marine currents also carry substantial amounts of waste, especially plastic debris, into the lower-lying areas of Cayo Blanco, where it accumulates heavily along surfaces reached by storm surges and tidal action (Fig. 3).

Sampling

Following years of near-total abandonment, dense vegetation has colonized both naturally accumulated sediments and areas filled with reclaimed materials, as well as modern and historical structures. Today, the vegetative cover represents a defining landscape element of the site, including in zones of archaeological interest such as the *Polvorín San Antonio* itself. In relation to the coexistence of anthropogenic and natural elements, Norwegian architect Christian Norberg-Schulz wrote in *Genius Loci*: *"We must nonetheless repeat that a place means more than just a location. Within the landscape, there are both subordinate places and natural*

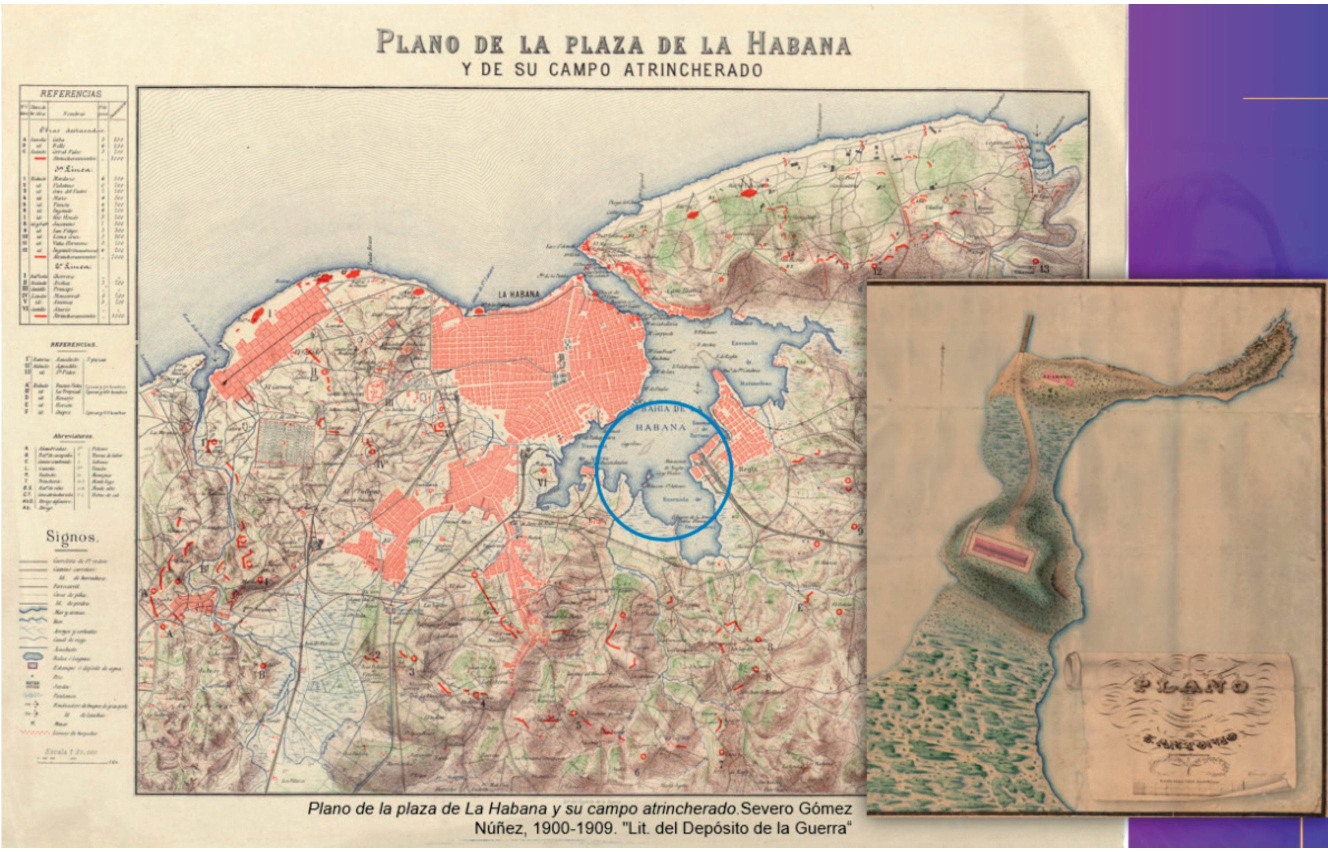

Figure 1. 1909 Map of Havana Showing the Location of the Cayo Blanco Site. In the map on the right, the outline of the *Polvorín* is clearly visible, along with the accumulation of fill material that led to the connection of the small island to the mainland.

Figure 2. Current Satellite Image from Google Earth with Overlay of the Former Islet of Cayo Blanco**.** The image also shows the Luyanó River and the adjacent industrial area extending toward the municipality of Regla.

things" (Norberg-Schulz, 1992). Building on this reflection, one may argue that in any site, even those characterized primarily by historical and archaeological features, vegetation can also be a defining component of the "spirit of place," or *Genius loci*. This intangible quality contributes to what makes a site unique and distinguishable from all others. In this sense, the plant component, often comprising well-defined phytocoenoses of ecological interest, should be considered part of the site's botanical heritage. Beyond its role in narrating the site's ecological and historical evolution, this component may include valuable species that deserve to be preserved and appreciated on par with historical and archaeological elements. These considerations underpin the multidisciplinary methodological framework adopted by the *Archeo-Cuba* project since its inception. Thus, alongside the collection of historical and archival data, and the study and cataloging of material evidence and findings from the target sites, the research phase also focused on the botanical elements present within the project areas. These elements were analyzed not only for their potential to support the interpretation of the site's historical stratigraphy but also to ensure their proper conservation and to explore their potential as cultural and tourist attractors, serving the broader functions and objectives of archaeologically significant areas. To achieve these aims, it was essential first to document the local flora and vegetation. In the specific case of Cayo Blanco, where no prior floristic or vegetation studies existed, field surveys were carried out with the support of local botanists to identify plant species. A floristic inventory was compiled and subsequently analyzed to distinguish native from exotic species, identify invasive taxa (Oviedo & González-Oliva, 2015), and highlight species of conservation interest, with reference to national protection lists (González-Torres et al., 2016).

Figure 3. Seedlings of *Avicennia germinans* (black mangrove) among debris carried by storm surges.

Results and Discussion

Floristic-vegetation analysis of the Cayo Blanco area

1. The floristic-vegetation survey of the area identified six main environmental contexts and corresponding vegetation formations:
2. Ruderal and exotic tree-shrub formations, mostly surrounding the *Polvorín San Antonio* and landfilled zones.
3. Relict patches of black mangrove (*Avicennia germinans*), occupying surfaces periodically submerged by tidal activity.
4. Sclerophyllous-dominated tree-shrub formations, growing on calcarenite outcrops near the tip of Cayo Blanco.
5. Pioneer native vegetation, including *Ficus citrifolia* and *F. crocata*, which has colonized built structures, including the *Polvorín San Antonio*.
6. Halophilic vegetation, found on open surfaces affected by wave action.
7. Ruderal herbaceous and weedy commensal vegetation, within remnants of agricultural land.

Understanding the species that compose these communities, their ecological roles, and biogeographic significance has enabled an initial reconstruction of the site's historical evolution. For instance, the ruderal communities rich in exotic species surrounding the *Polvorín San Antonio* and those in areas associated with industrial development indicate that these soils are primarily recent and anthropogenic in origin. Such substrates, formed largely by landfilling, are highly favorable to ruderal and invasive exotic species, which outcompete native flora, an interpretation also corroborated by historical cartography (Fig. 2). Among the many invasive species now established in Cuba, several have found ideal conditions for

Figure 4. *Leucaena leucocephala* a species native to Mexico and Central America, recognized by the IUCN's Invasive Species Specialist Group as one of the world's 100 most invasive species.
Figure 5. Agave offoyana, a native plant species occurring the Cayo Blanco area

growth and spread in the Cayo Blanco area. One of the most widespread and problematic is *Leucaena leucocephala* (Fig. 4), a species native to Mexico and Central America, recognized by the IUCN's Invasive Species Specialist Group as one of the world's 100 most invasive species.

This plant is abundant in the study area, along with other invasive exotics such as *Albizia lebbeck*, *Vachellia farnesiana*, *Ricinus communis*, *Mimosa pigra*, and *Schinus terebinthifolius*. These findings underscore how vegetation analysis can contribute to reconstructing the "historical stratigraphy" of a site. In this sense, the botanical elements present at Cayo Blanco should also be integrated into interpretive frameworks, enhancing the site's value as a cultural and touristic destination, alongside its archaeological, historical, and landscape features. In contrast, the portion of Cayo Blanco corresponding to the former island (Fig. 2) hosts sclerophyll-dominated vegetation, a relict plant community of significant documentary and conservation interest. Native to the site and now disappeared from the rest of Havana Bay, this community suggests that the area has been less disturbed and retains elements of Cuba's original flora. This vegetation is closely linked to the calcarenite substrate, which gives the site its name, *Cayo Blanco*. The species making up this phytocoenosis are all native, including *Ateleia cubensis, Exostema caribaeum*, *Opuntia stricta*, *Bourreria succulenta*, *Stigmaphyllon sagranum*, *Smilax havanensis*, *Crossopetalum aquifolium*, and *Agave offoyana* (Fig. 5) (García-Beltrán, 2024).

Exotic species are nearly absent here, in stark contrast to the other areas previously described. This provides insight into the typical zonal environments and the potential natural vegetation of this part of Havana Bay and is essential for defining the plant species to be used in future environmental restoration projects in the area. This aligns with the goals of the "*Plan Perspectivo de Desarrollo de la Bahía de La Habana"*, which includes reclaiming a substantial portion of the bay for public use, progressively shifting it away from industrial exploitation.

Another important plant community identified in the Cayo Blanco area is found in open surfaces subject to wave action and constant exposure to marine aerosol. These soils are characterized by high sodium chloride (NaCl) concentrations, selecting for halotolerant or halophytic species, which store water in their leaves. Notable examples include *Heliotropium curassavicum* and *Borrichia* × *cubana*. The latter species was previously only known from the northern coast of Havana, and Cayo Blanco now represents the first documented occurrence in this southern sector.

In front of the tip of Cayo Blanco lies a wetland area dominated by black mangrove (*Avicennia germinans*) (Fig. 6), forming a valuable mangrove stand. While such formations were likely common in Havana Bay historically, they have now been reduced to a few relict patches, making

Figure 5. *Agave offoyana,* a native plant species occurring the Cayo Blanco area

this remnant ecologically and historically significant. Mangroves, encompassing species of *Avicennia*, *Rhizophora*, and *Laguncularia,* are tropical and subtropical coastal trees. They are adapted to saline or brackish waters, tidal currents, strong winds, and muddy anoxic soils. Their survival in such extreme environments is facilitated by specialized structures such as pneumatophores: vertical aerial roots that emerge from the soil and absorb atmospheric oxygen to support respiration in oxygen-poor substrates (Fig. 6).

Mangroves also play a critical role in mitigating the effects of climate change. These dense coastal forests protect shorelines from wave erosion by stabilizing sediments with their trunks and root systems, encouraging the deposition of mud and sand. Additionally, organic matter from the mangroves, fallen leaves and dead branches, accumulates and helps form new landmasses. In the context of rising sea levels, this ecosystem service is especially crucial, as many coastal areas and small islands are increasingly vulnerable to submersion. For these reasons, there are now global efforts to restore and expand mangrove forests in high-risk coastal zones. Mangrove forests also function as biodiversity hotspots, serving as habitat and nurseries for a wide range of animal species (Guzmán Menéndez & Menéndez Carrera, 2006). Given this ecological and cultural importance, the mangrove fragment at Cayo Blanco should be prioritized for conservation and enhancement, including serving as a source of propagative material for establishing new mangrove stands within Havana Bay. Such efforts will require the development of a waste removal plan, as the area is currently inundated with debris. This cleanup must be carefully executed, as many viable *Avicennia germinans* seedlings are found among the refuse and deserve to be preserved and cultivated (Fig. 3). These seedlings, along with seeds and propagules of other native species such as *Thespesia populnea*, could form the basis for a pilot native plant nursery, supporting future restoration initiatives across the bay. Given the proximity of *Polvorín San Antonio* and the pre-Columbian sites of *Guasabacoa I and II,* both now threatened by sea-level rise, the expansion of the mangrove belt could also serve as a natural coastal buffer to protect these valuable archaeological assets. Such intervention would be a practical example of a Nature-Based Solution (NBS) within the Havana Bay area, directly aligned with one of the primary objectives of the *Archeo-Cuba* project: to identify and implement strategies to mitigate the adverse impacts of climate change on cultural heritage. Due to the site's low elevation, it is particularly vulnerable to sea-level rise, which exacerbates erosive processes, especially in the absence of natural barriers like mangroves.

Figure 6. Mangrove stand of *Avicennia germinans*

As part of the planning for a proposed Archaeological Park at Cayo Blanco, this NBS example should be accompanied by other ecological remediation strategies. Key among them is the reduction of the pollution coming from the nearby Río Luyanó, which empties near the tip of Cayo Blanco, and soil contamination throughout the bay, both legacies of industrial activity. These issues can also be addressed through targeted NBS approaches such as phytoremediation and constructed wetlands for wastewater treatment. A suitable wetland site for a future phytotreatment facility has already been identified on the opposite side of the river.

Botanical attractors: conservation and valorization

The floristic survey of the Cayo Blanco area has made it possible to identify plant species of greatest biogeographical and conservation interest, particularly those endemic to Cuba, as well as rare species (at local, regional, or national levels) and those included in national lists of protected plants, such as the Red Lists (González-Torres et al., 2016). By comparing the results of these investigations in Cuba with numerous scientific studies conducted at archaeological sites in Italy—especially within the Roman area—it becomes evident that the presence of a wide variety of plant species of conservation interest (Ceschin et al., 2006; Ceschin et al., 2012) is often a shared characteristic of places where traces of the past are preserved. This suggests that, beyond scientific relevance, the highlighting of rare, endemic, or protected plant species can also serve tourism development, turning these species into genuine cultural attractors. In addition, the study of the area's plant elements was extended to explore the compelling relationship between botany and cultural anthropology through the lens of ethnobotany. Traditional uses of the plant species identified at Cayo Blanco were investigated in the context of Cuban communities. These include medicinal, culinary, domestic, artisanal, apicultural, recreational, ritual-religious, and magical uses—knowledge documented in ethnobotanical research conducted across the island by Cabrera (1954), Roig (1974), Fuentes Fiallo (1999; 2001; 2002a; 2002b; 2003; 2004–2005; 2011–2012), Fuentes Fiallo and Castro (2000), Velázquez D. et al. (2014) and Carlomagno et al. (2015). A review of this rich bibliography reveals that many of the plant species recorded in the Cayo Blanco area are still in use today by the Cuban population. For instance, *Guazuma ulmifolia*, known locally as "guasima" (Roig, 2014), is used for its timber; others serve culinary purposes, such as *Portulaca oleracea* and *Phyllostylon rhamnoides*; but it is especially in the medicinal realm that ethnobotanical knowledge is most richly represented. Medicinal plants include *Koanophyllon villosum, Cynophalla flexuosa, Turnera ulmifolia, Varronia globosa* (known as yerba de la sangre), *Turnera ulmifolia* (marilope), and *Morinda royoc* (raíz de indio). A significant number of species recorded in the study area have recognized ethnomedicinal applications (Roig, 1974), and a full list is provided in Table 1.

Table 1 - Species with ethnomedicine uses recorded in Cayo Blanco.

Agave offoyana Jacobi
Avicennia germinans (L.) L.
Cecropia schreberiana subsp. *antillarum* (Snethl.) C. C. Berg & P. Franco
Cynophalla flexuosa (L.) J. Presl
Exostema caribaeum (Jacq.) Roem. & Schult.
Guazuma ulmifolia Lam.
Heliotropium angiospermum Murray
Koanophyllon villosum (Sw.) R. M. King & H. Rob.
Lantana aculeata L.
Leucaena leucocephala (Lam.) de Wit
Morinda royoc L.
Petiveria alliacea L.
Thespesia populnea (L.) Sol. ex Corrêa
Turnera ulmifolia L.
Vachellia farnesiana (L.) Wight & Arn.
Varronia globosa Jacq.
Viguiera dentata (Cav.) Spreng.

Figure 7. *Ficus citrifolia* along the walls of the Polvorín San Antonio

In light of these findings, the Cayo Blanco–Guasabacoa area emerges as a compelling model of interdisciplinary integration, where elements of historical and archaeological heritage are closely interwoven with botanical and ethnobotanical components, contributing to the creation of a richer, more layered cultural and educational offering. This setting serves as an exemplary case of how history, archaeology, nature, and plant knowledge can be effectively combined, enhancing the site's cultural value and broadening its potential as a resource for education, tourism, and community engagement.

Management Plan for the Cayo Blanco Area

The results of the floristic–vegetational and ethnobotanical studies, combined with the historical and archaeological knowledge gained largely through the investigations carried out under the Archeo-Cuba Project, were fundamental to the development of a Management Plan for the Cayo Blanco area, aimed primarily at the appropriate conservation and valorization of the site. The Plan, developed collaboratively with archaeologists from the Oficina del Historiador de la Ciudad de La Habana and planners from the Ministerio de la Construcción also included the reuse and reinterpretation of residual or abandoned structural elements onsite, such as reinforced concrete blocks, iron beams, and sheet metal. This approach is emblematic of the Archeo-Cuba methodology, which seeks to valorize even these modern remnants as part of the site's recent historical narrative.

The Plan further incorporates the enhancement of the site's agricultural landscape, especially the section near the entrance bordered on the left by the Luyanó River. This area is currently used for the cultivation of edible plants such as banana (*Musa* × *paradisiaca*), Cuban oregano (*Plectranthus amboinicus*), cassava (*Manihot esculenta*), and sweet potato (*Ipomoea batatas*). This zone lies on an alluvial substrate, deeper, moist, and fertile, making it particularly suitable for agriculture. From a design standpoint, and in light of the educational potential of the Cayo Blanco site, it is essential to preserve, expand, and enhance this cultivated strip, not only to familiarize visitors with local crops but also to emphasize the importance of agrobiodiversity conservation in Cuba, and to highlight the relevance of self-production practices, especially critical in a context heavily burdened by the economic embargo. With respect to the native pioneer vegetation, particularly *Ficus citrifolia* and F. *crocata*, which have colonized the masonry of the Polvorín San Antonio (Fig. 7), a site of great historical and archaeological interest, management decisions must balance conservation needs and ecological realities.

While these plants may represent a threat to the structural integrity of the architecture, complete removal is not recommended, particularly for mature individuals, for two main reasons:

Structural concern: Simply cutting back large specimens would not prevent regrowth. Effective removal would require chemical herbicides such as glyphosate or triclopyr. However, the subsequent decomposition of the embedded root biomass could destabilize the masonry, posing a serious risk to the structural conservation of the building.

Cultural–ecological value: In line with the "conservative approach" adopted by the Archeo-Cuba Project, the natural evolution of vegetation is considered part of the site's history and spirit. Even recent plant colonization processes can serve as indicators of the genius loci, contributing to a layered reading of the site over time.

Conclusions

The Archeo-Cuba Project has offered a valuable opportunity for international collaboration and capacity-building focused on the relationship between cultural heritage and sustainable intervention strategies in areas vulnerable to climate change. Italian expertise, widely recognized for its integrated approach to cultural heritage, from natural landscapes to anthropized environments, has supported interdisciplinary exchange, particularly through the educational program and on-site workshops carried out with Cuban professionals. Specifically, the floristic–vegetational and ethnobotanical studies conducted in the Cayo Blanco area, along with the historical and archaeological findings largely derived from the same project, have contributed not only to enriching the site's cultural offering but also to addressing key environmental and management challenges. These studies were essential in the drafting of the Cayo Blanco Management Plan, whose primary goal is the proper stewardship and enhancement of the site. An important final output of the project will be the definition of guidelines for replicating the Archeo-Cuba model, already considered a best practice with the potential to be scaled and adapted to other Cuban heritage sites. The results of these georeferenced surveys have been integrated into a Web-GIS platform (https://webgis.archeocuba.com/), developed as a technological hub for project information and content—open-access and consultable, supporting further research, monitoring, and decision-making.

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References

Cabrera, L. (1954). El Monte. *Ediciones Universal*.

CANEVA, G. (2005). La biologia vegetale per i beni culturali, conoscenza e valorizzazione, vol. II. *Edizioni Nardini, Firenze*.

Carlomagno, A., Pardini, A., & Esquijerosa, Y. C. (2015). Medicinal plants in ethnobotanical and religious traditions in Cuba: A first review and updating. *Associazione Scienze Agrarie Tropicali: Ragusa, Italy*, 21.

Ceschin, S., Cancellieri, L., Caneva, G., & Battisti, C. (2012). Size area, patch heterogeneity and plant species richness across archaeological sites of Rome: Different patterns for different guilds. *Vie et Milieu - Life and Environment*, *62*(4), 165–171.

Ceschin, S., Caneva, G., & Kumbaric, A. (2006). Biodiversity and floristic emergencies in Roman archaeological areas. *Webbia*, *61*(1), 133–144.

Cicinelli, E., Salerno, G., & Caneva, G. (2018). An assessment methodology to combine the preservation of biodiversity and cultural heritage: the San Vincenzo al Volturno historical site (Molise, Italy). *Biodiversity and conservation*, *27*(5), 1073-1093.

Digna Velázquez, V. D., Guyat Dupuy, M. A., Manzanares Ayala, K., Aguirre Dorado, B., & Gelabert Ayon, F. (2014). Etnobotánica: Empleo de plantas para uso medicinal. *Revista Cubana de Ciencias Forestales*, *2*(1).

Fiallo, V. R. F. (1999). Apuntes para la flora económica de Cuba I. Especies productoras de fibras. *Revista del Jardín Botánico Nacional*, *20*, 57-82.

Fiallo, V. R. F. (1999). Apuntes para la flora económica de Cuba II. Plantas galactógenas. *Revista del Jardín Botánico Nacional*, *20*,83-90.

Fiallo, V. R. F. (2001). Apuntes para la flora económica de Cuba IV. Especies medicinales de uso veterinario. *Revista del Jardín Botánico Nacional*, *22*(2), 221-245.

Fiallo, V. R. F. (2002). Apuntes para la flora económica de Cuba V: Plantas tintóreas. *Revista del Jardín Botánico Nacional*, *23*(1), 91–113.

Fiallo, V. R. F. (2002). Apuntes para la flora económica de Cuba VI: Especies cauchógenas. *Revista del Jardín Botánico Nacional*, *23*(2), 255–262.

Fiallo, V. R. F. (2003). Apuntes para la flora económica de Cuba VII: Especies frutales. *Revista del Jardín Botánico Nacional, 24*(1/2), 177–217.

Fiallo, V. R. F. (2004–2005). Apuntes para la flora económica de Cuba VIII: Especies ceríferas. *Revista del Jardín Botánico Nacional, 25/26*, 161–165.

Fiallo, V. R. F. (2011). Apuntes para la flora económica de Cuba IX: plantas para cercas y setos vivos. *Revista del Jardín Botánico Nacional*, 205-228.

Fiallo, V. R. F., & López Castro, L. P. (2000). Apuntes para la flora económica de Cuba III: Plantas condimenticias. *Revista del Jardín Botánico Nacional, Universidad de la Habana*, *21*, 47–70.

Fiallo, V. R. F., & Castro, L. P. L. (2000). Apuntes para la flora económica de Cuba III. Plantas condimenticias. *Revista del Jardín Botánico Nacional*, *21(*1), 47-70.

García-Beltrán, J.A. 2024. *Fabaceae.*En: GEPC. Catalogo de las Plantas de Cuba. Planta! –Plantlife Conservation Society, Vancouver, 498-562.

González-Torres, L. R., Palmarola, A., Barrios, D., González-Oliva, L., Testé, E., Bécquer, E. R., ... & Granado, L. (2016). Estado de conservación de la flora de Cuba,. *Bissea*, *10*(1), 1-23.

Guzmán Menéndez, J. M., & Menéndez Carrera, L. (2006). Ecosistema de manglar en el archipiélago cubano. Sello Editorial Academia.

Norberg-Schulz, C. (1992). Genius loci - Paesaggio, ambiente, architettura (Documenti di architettura). Electa.

Oviedo, R., & González-Oliva, L. (2015). Lista nacional de plantas invasoras y potencialmente invasoras en la República de Cuba-2015, *Bissea*, 1-88.

Ricciardi, M. A., & Salerno, G. (2024). Archeo-Cuba: archeologia e sostenibilità ambientale. Studio e conoscenza degli aspetti storici, archeologici e naturalistici finalizzati ad una gestione eco-sostenibile in siti cubani / Archeo-Cuba: archaeology and environmental sustainability. Study and knowledge of historical, archaeological and naturalistic aspects aimed at eco-sustainable management in Cuban sites. *Il Capitale Culturale. Studies on the Value of Cultural Heritage*, (29), 169–191.

Roig, J. T. (1974). Plantas medicinales, aromáticas o venenosas de Cuba-2. *Editorial Ciencia y Técnica*.

Roura Alvarez, L. (n.d.). Arqueología, cooperación y sostenibilidad: Estrategia de futuro. *Revista del Gabinete de Arqueología*, *15*(15).

Viera, D. V., Dupuy, M. A. G., Manzanares, K., Dorado, B. A., & Ayon, F. G. (2014). Etnobotánica: Empleo de plantas para uso medicinal. *Revista Cubana de Ciencias Forestales: CFORES*, *2*(1), 4.

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ON THE TIME TREND OF AEROBIOTIC AND ANTHROPIC BIOMASS: AN APPROACH TO THE UNKNOWN

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Abstract – Biodiversity changes in the aerobic biomass have been estimated (including the "anthrobiome"). These changes seem to be strongly affected by the anthropic impact along a long-time span (starting from 2,060 AD to 12,060 BC). The aerobic biomass excluding *Homo sapiens* shows a progressive and, perhaps, unstoppable decline, after the advent of Western industrial civilization. This preliminary work aims to highlight how biodiversity should perhaps be studied, understood and used critically by adopting innovative approaches (e.g., using biomass) and without preconceptions. Although our work is based on assumptions regarding some parameters that have been widely approximated, we believe it is necessary to proceed with this exploratory analysis considering the dramatic decline in biodiversity worldwide.

Keywords: anthrobiome, Biomass, Aerobios, simpson index

Introduction

Biodiversity—albeit in a symbolic and ante litteram form—has been admired and respected since the dawn of human history. From its veneration in Eastern spiritual traditions (e.g., Hinduism, Buddhism), to the first conceptual frameworks proposed by Aristotle, to the vivid allegories of Lucretius and the mystical interpretations of St. Francis of Assisi; from the early formal naming systems (e.g., Cesi, 1651), to Linnaeus's foundational taxonomic system (1758), and the evolutionary insights of Darwin (long before the official publication in 1859) and Wallace (1858); through the quantitative formulations influenced by thermodynamics and information theory—Gini (1912), Shannon (1948), Wiener (1948), Simpson (1949), Margalef (1957), Hill (1973), Whittaker (1972, 1977), and Patil and colleagues (Patil & Taillie, 1976; Grassle et al., 1992); to the unconventional but constructive critiques of Hurlbert (1971); the qualitative-quantitative approaches of May (1975); and finally, its establishment as a distinct scientific discipline, notably with Wilson (1992) and many others (e.g., Alatalo, 1981; Barbault et al., 1991; Colwell, 1979; Ganis, 1991; Magurran, 2004; Odum, 1973; Pielou, 1975; AA.VV., 2000).

Biodiversity has since been embraced—though often more in rhetoric than in practice, and not without risks of conceptual distortion (Battisti & Contoli, 2011)—by international political discourse (e.g., Barton, 1992).

The current crisis of biodiversity in the biosphere has long been highlighted at various levels, especially at the taxonomic level (e.g., Wilson, 1992; Gaston & Mound,

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1993). However, given the current, ongoing uncertainty about the numerousness of taxa (in particular, species) and the respective abundance of individuals, complete knowledge of the biodiversity in the biosphere remains unknown (e.g., Mora et al., 2011; Caley et al., 2014; Hortal et al., 2015; Guedes et al., 2025). An approach to 'complex biodiversity', that is, an extended concept of biological diversity that simultaneously takes into account the various conceptual components (abundance, richness, diversity etc.), seems easier by examining biomass, large eco-environmental units and their respective dominant and characterizing taxa, as they are at the core of the functional relationships within and among ecosystems (Daly et al., 2018). This could allow a more realistic approach than the strictly taxonomic one, from an energetic and eco-functional point of view (see Barnes et al., 2018; Allen et al., 2002).

It is important to distinguish between the concepts of ecosystem and biome. An ecosystem is linked to a territorially continuous biotope and characterized by a specific biotic composition. A biome, by contrast, is defined primarily by abiotic factors—such as macroclimate—which allows it to include ecologically similar but geographically distant and disconnected ecosystems (Clapham, 1973).

This broader view supports those (e.g., Ellis & Ramankutty, 2007) who define biomes as including human-modified areas, where a single species—*Homo sapiens*—profoundly alters biogeochemical cycles: from soil potential and food production at all trophic levels to consumption, elimination of trophic competitors, and the exploitation of local biotic and abiotic resources. This leads to the concept of the Anthrobiome (Couvet, 2019). The biomass from Anthrobiomes, together with that from natural biomes, forms an extended biomass, which can be studied in a diachronic perspective across millennia.

Furthermore, the anthropogenic impact—recognized as a major driver of ecological change—must be integrated into analyses. This impact differs qualitatively between the halobios (marine organisms), where human influence is still mainly through extraction and pollution, and the aerobios (terrestrial organisms), where humans have long replaced natural ecosystems with anthroposystems based on agriculture, livestock, and settlement (Machlis et al., 1997; Likhacheva et al., 2019). In terrestrial systems, humans have become a dominant ecological force and a major threat to biodiversity (Battisti et al., 2016).

Such an analysis can now rely on robust time-resolved data concerning human population trends since the Holocene, the characteristics of major aerobios biomes, and the distribution of biomass across biological organisms—including *Homo sapiens*—despite the need for substantial numerical approximations in these estimates (Wilkes et al., 2025). Accordingly, we propose to examine biodiversity through the lens of biomass distribution within the aerobios, using diversity indices calculated on the biomass frequency of eco-taxo-physiognomic groups within each biome, rather than on individual abundance. In this framework, eco-taxo-physiognomic groups are defined by dominant plant physiognomies (e.g., conifers, deciduous broadleaf trees, evergreen broadleaf trees, etc.).

Although this approach shows some weaknesses due to the high degree of approximation related to the coarse-grained method, we think that the general patterns may be objectively credible. We think also that this type of analyses is urgent due to the strong impact of *Homo sapiens* in the biosphere.

Methods

Data base

Biomass data, expressed in gigatonnes of carbon (Gt C), were sourced from Bar-On et al. (2018). The time span analyzed extends over the past 10,000 years—starting from the onset of a more stable macroclimate and the rise of agriculture and livestock, which triggered the ongoing expansion of *Homo sapiens*. The projection extends 55 years into the future (UN, 2019), with a forecasted global population of approximately 10 billion individuals (1010). For clarity in graphical representation, time is expressed as Log(12,060 – years), spanning from 12,060 BCE to 2,060 CE. The analysis focuses on several major biomes for which consistent and reliable data are available (Lieth, 1975; Loidi et al, 2022).

The following parameters have been considered over time: the number of individuals of *Homo sapiens*; its average individual biomass, assumed as 50 kg per capita, with approximation (Walpole et al., 2012); the current biomass of the main eco-taxo-physiognomic groups, i.e., the main plant physiognomies (Bar-On et al., 2018; Trost, 2022).

We obtained the numerical proportions of biomass between the aforementioned groups from Lieth (1975) and the human biomass or "anthrobiome" as the product of the population in a given year and the weight of a single individual of *Homo sapiens* (Groombridge, 1992). We proposed an indicative evaluation of biodiversity across different biomes, based on the biomass of eco-taxo-physiognomic groups. Given the impossibility of quantifying the biomass of every taxonomic component within each biome, we focused on identifying the dominant taxa in terms of biomass, considering only tree species. A total of 104 species was selected as a reference: specifically, 104 species for tropical rainforest, 103 for seasonal rainforest, 102 for temperate, seasonal, and evergreen sclerophyllous scrub, and 101 for Taiga. In addition, we included herbaceous plants sensu lato in the "prairie" biome (104 species), lichen symbioses in the Tundra (101 species), and various plant species that occur predominantly in isolated, scattered, and individual forms in the "semi-desert" biome (101 species), following the approach outlined in the seminal work by Cazzolla Gatti et al. (2022). Finally, we obtained the Taxonomic diversity (TAX D; Cazzolla Gatti et al. 2022).

Before 1975 and after 2018, the values were tentatively estimated to the dates 10,000 (approximately, the beginning of agriculture and livestock farming), 7,000, 5,000, 3,000 B. C. and 1500 (end of the Middle Ages), 1800 ("industrial revolution"), 1950 ("green revolution") B. C., as well as to 2055 (Homo sapiens with 1010 presumed individuals; UN, 2019).

Starting from the data of Bar-On et al. (2018), we calculated (i) the biomass and the relative frequencies of the various biomes and (ii) biomass of 'anthrobioma'. From these biomass values, we obtained: the total biomass for aerobios (including anthrobioma; BIOM; from Bar-On et al., 2018); the fraction of Homo sapiens biomass (on the total aerobios biomass; H BIO; calculated as the number of individual humans

Table 1. Data of biomass and diversity along the time span considered. Biomass is expressed in gigatonnes of Carbon (GT of C). Time considered, log-transformed, started from the present up to ten millennia ago. The biomes considered are from Lieth (1975). Acronyms: BIOM: total biomass for aerobios (Bar-On et al., 2018); H BIO: Fraction of *Homo sapiens* Biomass (calculated as the number of individual humans multiplied by 50 kg: Walpole et al., 2012); TAX D: Taxonomic diversity (Cazzolla Gatti et al. 2022); BIO D: Biomic diversity (Magurran, 2004). Biome acronyms: ANT: Anthrobioma; PLU: pluvial/tropical forests; PLU S: seasonal pluvial/tropical forests; TEM: temperate *sensu lato*; TEM S: seasonal temperate; SCL: sclerophilous forests; TAI: taiga; PRA: prairies; TUN: tundra; DES: semi-desertic biome.

Year	10000 B.C.	7000 B.C.	5000 B.C.	3000 B.C.	1500 A.D.	1800 A.D.	1950 A.D.	1975 A.D.	2018 A.D.	2055 A.D.
LOG Y	5.919	6.043	6.151	6.296	7.252	7.585	7.921	8.022	8.377	9.3
BIOM	589	588	587	586	585	584	577	567	541	474
H BIO	0.0006	0.0006	0.0006	0.0006	0.0006	0.0061	0.0071	0.0151	0.0043	0.022
TAX D	0.999	0.999	0.999	0.999	0.999	0.994	0.993	0.985	0.997	0.978
BIO D	0.681	0.681	0.681	0.674	0.674	0.695	0.707	0.721	0.36	0
PLU	312	312	312	312	312	288	279	267	231	163
PLU S	72	72	72	72	72	71	70	68	66	48
TEM	33	32	31	30	29	28	27	26	25	23
TEM S	37	37	37	37	37	37	37	36	35	33
SCL	5	5	5	5	5	5	5	5	5	5
TAI	43	43	43	43	43	43	43	42	41	39
PRA	101	101	101	101	101	101	100	98	95	84
TUN	5	5	5	5	5	5	5	5	5	5
DES	5	5	5	5	5	5	5	5	5	5

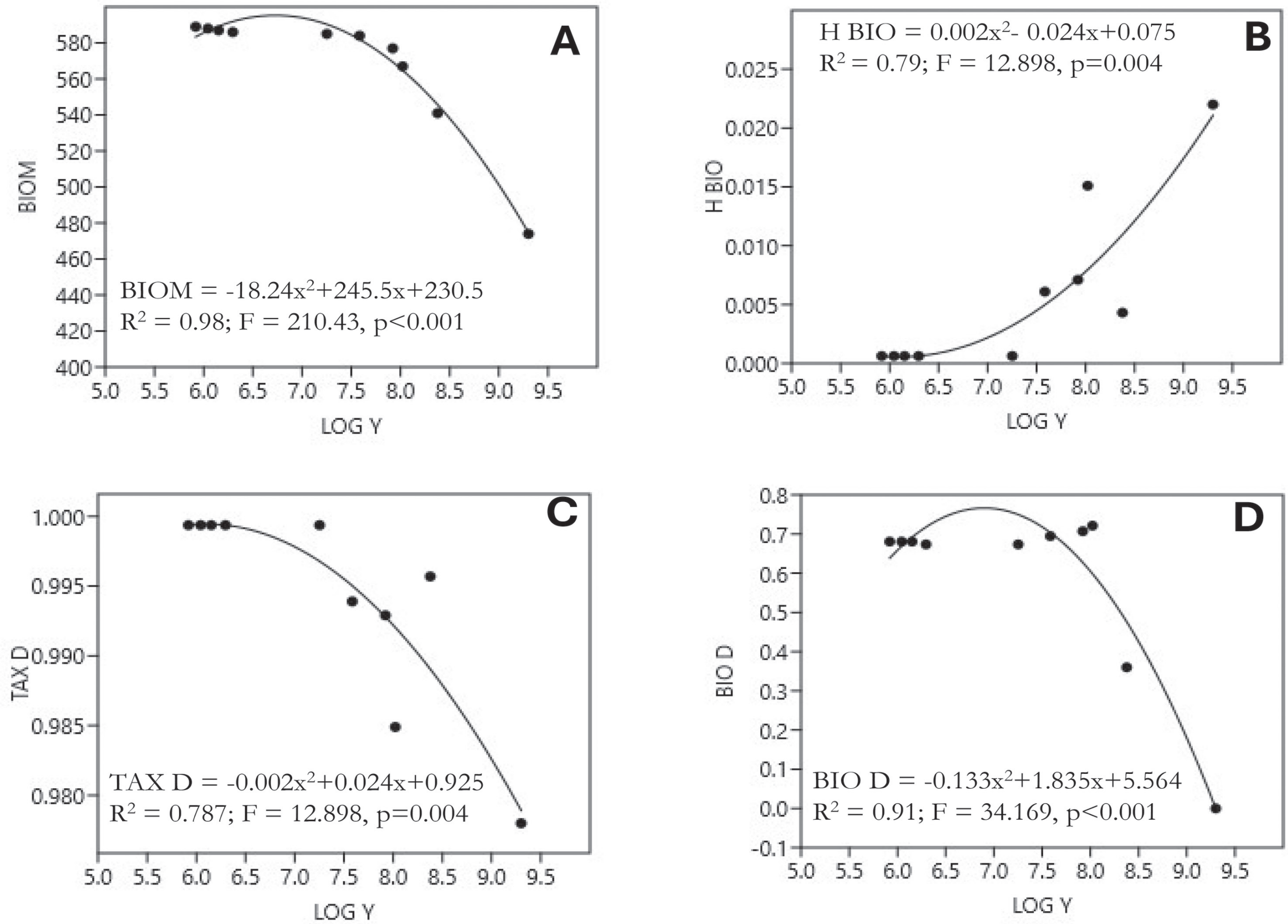

Figure 1. Trends along the time range studied (log y=log years after 12.060 B.C.; see Methods for details) with equations of polynomial regression, order 2. A: total biomass for aerobios (including anthrobioma; BIOM); B: Fraction of *Homo sapiens* Biomass (on the total aerobios Biomass; H BIO); C: Taxonomic diversity (TAX D); D: Biomic diversity (BIO D).

multiplied by 50 kg: Walpole et al., 2012). Moreover, from the biomass values for each biome (including anthrobiome), we obtained the relative frequency (fr) of biomass for each biome. After, we calculated the Simpson diversity index as $D=1-\Sigma fr^2$ (Magurran, 2004; see also Patil & Taillie, 1976; Ganis, 1991; Contoli Amante & Luiselli, 2015). The Simpson index has also been calculated on the biomass of the biomes (included the anthrobiome) divided by the number of species, obtaining an estimate of the average biomass per species of the arboreal layer (or more in general of the dominant species; Bar-On et al, 2018; biomic diversity: BIO D; Magurran, 2004). The trend over time of the overall aerobic biomass and of diversity index was therefore verified.
We correlated BIOM, H BIO, TAX D, and BIO D to the range studied (log y=log years after 12.060 B.C, performing equations of polynomial regression, order 2 and using the PAST 4.01 software (Hammer et al. 2001). Alpha was set to 0.05 level.

RESULTS AND DISCUSSION

Trend in all the values of biomass (including anthropic biomass or 'anthrobiome') and of the Simpson diversity index have been reported in Table 1 and Fig. 1.
We observed a progressive decline of the total biomass (Fig. 1A) largely due to the decline in biomass of the aerobios, while anthropic biomass increased progressively (Fig 1B).
The taxonomic biodiversity (i.e., the numerousness of species, Fig. 1C) has always been very high in the temporal range considered (approximately 107 species). However, over time, the biodiversity shows, after an initial stability, a progressive decline, due to the growing role of the biome dominated by a single species (Homo sapiens) towards the others.
The biomic biodiversity (Fig 1D) after a very slight initial decline, shows an increase, as expected, given the growing weight of the anthrobiome, initially quantitatively negligible

and today tendentially hegemonic over the others. The future forecast (2055) is that of a rapid collapse, if the current trends were to be confirmed. Our data support evidence yet provided by other authors (Pereira et al., 2010, Segan et al., 2016) with implication for cascade effects also on our species (Undheim & Ahmad, 2024).
Our preliminary data indicate a decline in total biomass, which appears to contrast with the progressive increase in anthropic biomass. This reflects a clear anthropogenic pressure on the aerobios—and thus on the biosphere as a whole. The onset of this decline seems to coincide with the historical phase spanning from the discovery of the Americas to the beginning of the Euro-Western Industrial Revolution
This preliminary work aims to emphasize the need to study, interpret, and apply the concept of biodiversity critically and without preconceptions—as an essential tool for understanding biological reality. While our analysis is based on certain assumptions and approximate parameters subject to a degree of uncertainty (see Ladle, 2009), we consider this exploratory approach necessary given the alarming global decline in biodiversity.

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Foreword

This work was born from an idea of Longino Contoli, who recently passed away (2024). To him, friend and Magister, we dedicate our effort to make it public. The language and style of the work may seem outdated, but we wanted to keep it because it is characteristic of this researcher trained in the 70s when the way of writing articles was different from today. We wanted to interpret the original content as closely as possible.

References

AA. VV. (2000). Biodiversity. *Nature 405*, 207-253.

Allen, A. P., Brown, J. H., & Gillooly, J. F. (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science, 297(*5586), 1545-1548.

Barbault, R., Colwell, R. K., Dias, B., Hawksworth, D. L., Huston, M., Laserre, P., ... & Younes, T. (1991). Conceptual framework and research issues for species diversity at the community level. *From genes to ecosystems: a research agenda for biodiversity. IUBS, Cambridge, Mass*, 37-71.

Barnes, A. D., Jochum, M., Lefcheck, J. S., Eisenhauer, N., Scherber, C., O'Connor, M. I., ... & Brose, U. (2018). Energy flux: the link between multitrophic biodiversity and ecosystem functioning. *Trends in ecology & evolution*, *33*(3), 186-197.

Bar-On, Y. M., Phillips, R., & Milo, R. (2018). The biomass distribution on Earth. *Proceedings of the National Academy of Sciences*, *115*(25), 6506-6511.

Barton, J. H. (1992). Biodiversity at Rio. *BioScience*, *42*(10), 773-776.

Battisti, C., & Contoli, L. (2011). Diversity indices as 'magic'tools in landscape planning: a cautionary note on their uncritical use. *Landscape Research*, *36*(1), 111-117.

Battisti, C., Poeta, G., & Fanelli, G. (2016). An introduction to disturbance ecology. *Cham: Springer*, 13-29.

Caley, M. J., Fisher, R., & Mengersen, K. (2014). Global species richness estimates have not converged. *Trends in Ecology & Evolution*, *29*(4), 187-188.

Cazzolla Gatti, R., Reich, P. B., Gamarra, J. G., Crowther, T., Hui, C., Morera, A., ... & Liang, J. (2022). The number of tree species on Earth. *Proceedings of the National Academy of Sciences*, *119*(6), e2115329119.

Cesi, F. (1651). Plantarum inter se diversitas. Tabulae phytosophicae, XVIIII. In : 'Thesaurus Mexicanus'.

Clapham Jr. W.B. (1973). Natural ecosystems. MacMillan, New York.

Colwell, R. K. (1979). Toward a unified approach to the study of species diversity. *Ecological diversity in theory and practice. International Cooperative Publishing House, Fairland, Maryland, USA*, 75-91.

Contoli Amante, L., & Luiselli, L. (2015). Contributions to Biodiversity Theory: the importance of Formal Rigor. *Web Ecology*, *15*(1), 33-37.

Couvet, D. (2019). Citizen and Landscape Governance. In *Current Trends in Landscape Research* (pp. 287-302). Cham: Springer International Publishing.

Daly, A. J., Baetens, J. M., & De Baets, B. (2018). Ecological diversity: measuring the unmeasurable. *Mathematics*, *6*(7), 119.

Darwin C. (1859). On the origin of species by means of natural selection (Vol. 167). John Murray, London.

Ellis, E. C., & Ramankutty, N. (2008). Putting people in the map: anthropogenic biomes of the world. *Frontiers in Ecology and the Environment*, *6*(8), 439-447.

Ganis, P. (1991). La diversità specifica nelle comunità ecologiche: concetti, metodi e programmi di calcolo. *Quaderni del Gruppo Elaborazione Automatica Dati Ecologia Quantitativa, Dipartimento di Biologia, Università di Trieste, CETA (International Center of Theoretical and Applied Ecology), GEAD-EQ*, (10), 97.

Gaston, K. J., & Mound, L. A. (1993). Taxonomy, hypothesis testing and the biodiversity crisis. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *251*(1331), 139-142.

Gini, C. (1912). *Variabilità e mutabilità: contributo allo studio delle distribuzioni e delle relazioni statistiche.[Fasc. I.]*. Tipogr. di P. Cuppini.

Grassle, J.F., Patil, G. P., Smith, W., Taillie, C. (Eds) (1992). Ecological Diversity in Theory and Practice. *International Cooperative Publishing House*, Fairland, MD.

Groombridge, B. (1992). Status of the Earth's living resources. *Global Biodiversity*.

Guedes, J. J., Moura, M. R., Jardim, L., & Diniz-Filho, J. A. F. (2025). Global patterns of taxonomic uncertainty and its impacts on biodiversity research. *Systematic Biology*, syaf010.

Hammer, Ø., & Harper, D. A. T. (2001). Past: Paleontological Statistics Software Package for Educaton and Data Anlysis. *Palaeontologia Electronica*, *4*(1), 1-9.

Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. *Ecology*, *54*(2), 427-432.

Hortal, J., De Bello, F., Diniz-Filho, J. A. F., Lewinsohn, T. M., Lobo, J. M., & Ladle, R. J. (2015). Seven shortfalls that beset large-scale knowledge of biodiversity. *Annual review of ecology, evolution, and systematics*, *46*(1), 523-549.

Hurlbert, S. H. (1971). The Nonconcept Of Species Diversity: A Critique and Alternative Parameters. *Ecology*, *52*(4), 577-586.

Ladle, R. J. (2009). Forecasting extinctions: uncertainties and limitations. *Diversity*, *1*(2), 133-150.

Lieth, H. (1975). Primary productivity in ecosystems: comparative analysis of global patterns. In: *Unifying Concepts in Ecology: Report of the plenary sessions of the First international congress of ecology, The Hague, the Netherlands, September 8–14, 1974* (pp. 67-88). Dordrecht: Springer Netherlands.

Likhacheva, E. A., Nekrasova, L. A., & Kladovschikova, M. E. (2019). Geomorphic assessment of terrestrial resources. *Geography, Environment, Sustainability*, *12*(2), 78-86.

Loidi, J., Navarro-Sánchez, G., & Vynokurov, D. (2022). Climatic definitions of the world's terrestrial biomes. *Vegetation Classification and Survey*, *3*, 231-271.

Machlis, G. E., Force, J. E., & Burch Jr, W. R. (1997). The human ecosystem part I: the human ecosystem as an organizing concept in ecosystem management. *Society & Natural Resources*, *10*(4), 347-367.

Magurran A., 2004. Measuring biological diversity. Blackwell Publishing Malden, MA.

May, R. M. (1975, May). Stability in ecosystems: some comments. In *Unifying Concepts in Ecology: Report of the plenary sessions of the First international congress of ecology, The Hague, the Netherlands, September 8–14, 1974* (pp. 161-168). Dordrecht: Springer Netherlands.

Minerbi, S., & Cescatti, A. (2015). Tree volume and biomass equations for Picea abies and Larix decidua in South Tyrol. *For. Obs.*, *7*, 5-34.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G., & Worm, B. (2011). How many species are there on Earth and in the ocean? *PLoS biology*, *9*(8), e1001127.

Patil, G. P., & Taillie, C. (1976, August). Ecological diversity: concepts, indices and applications. In *Proceedings of the 9th Int. biometric conference, The Biometric Society* (Vol. 2, pp. 383-411).

Pereira, H. M., Leadley, P. W., Proença, V., Alkemade, R., Scharlemann, J. P., Fernandez-Manjarrés, J. F., ... & Walpole, M. (2010). Scenarios for global biodiversity in the 21st century. *Science*, *330*(6010), 1496-1501.

Segan, D. B., Murray, K. A., & Watson, J. E. (2016). A global assessment of current and future biodiversity vulnerability to habitat loss–climate change interactions. *Global Ecology and Conservation*, *5*, 12-21.

Shannon, C. E. (1948). A mathematical theory of communication. *The Bell system technical journal*, *27*(3), 379-423.

Simpson, E. H. (1949). Measurement of diversity. *Nature*, *163*(4148), 688-688.

Trost P., (2022). Quanto è attendibile l'ipotesi di 1000 miliardi di alberi? *Natura e Società 3,* 11-13.

Undheim, T. A., & Ahmad, T. (2024). Quantitative scenarios for cascading risks in AI, climate, synthetic bio, and financial markets by 2075. *Frontiers in Complex Systems*, *2*, 1323321.

United Nations (2019). World Population Prospects 2019, Online Edition. *Department of Economic and Social Affairs, Population Division*, New York.

Wallace, A. (1858). On the tendency of varieties to depart indefinitely from the original type. *J. Linn. Soc. Lond. Zool.*, *3*, 53-62.

Walpole, S. C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., & Roberts, I. (2012). The weight of nations: an estimation of adult human biomass. *BMC public health*, *12*(1), 439.

Whittaker, R. H. (1972). Evolution and measurement of species diversity. *Taxon*, *21*(2-3), 213-251.

Whittaker R.H. (1977). Evolution of species Diversity in land communities. *Evolutionary Biology, 10,* 1-67.

Wiener, N. (1948) Cybernetics: Control and Communication in the Animal and the Machine. *John Wiley & Sons*, New York, 194.

Wilkes, M. A., Mckenzie, M., Johnson, A., Hassall, C., Kelly, M., Willby, N., & Brown, L. E. (2025). Revealing hidden sources of uncertainty in biodiversity trend assessments. *Ecography*, *2025*(5), e07441.

Wilson E. O. (1992). The diversity of life. Harvard University press, Cambridge, Massachusetts.

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EVALUATION OF SUNFLOWER CULTIVARS TO DROUGHT STRESS IN DIFFERENT STAGES OF PHENOLOGY

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Abstract - In order to evaluate sunflower cultivars under drought stress, an experiment was conducted in 2024 on four sunflower cultivars in split plots in a randomized complete block design with 3 replications at the West Azerbaijan Research Center, Khoy. Drought stress treatment was applied at four levels in the main plots including: no water stress, stress at tillering stage, stress at flowering stage, stress at grain filling stage, and hybrids, Euroflor, Azargol and open-pollinated cultivars Master and Lakomka were considered in subplots. The results showed that the effect of main and sub-factors on agronomic traits and also The interaction effect of drought stress × cultivar on grain yield, biological yield, and harvest index was significant at the ($P \leq 0.01$), As the Azargol variety, in the presence of stress in head formation, reduces its yield by 9.68%, while the Euroflor variety shows a 18.24% decrease. Alsot the Azargol cultivar, in the presence of flowering stress, reduces its yield by 28.57%, while the Euroflor cultivar shows a reduction of 37.17%. Finally, the Azargol cultivar reduces its yield by 18.86% in the presence of grain filling stress, while the Lakumka cultivar showed a 25.64% reduction. Also, in the presence of stress, the Master cultivar showed the lowest reduction in harvest index at the stage of head formation and grain filling (7.08% and 5.25%, respectively) compared to the Euroflor cultivar (10.94%) and (8.31%), and the Lakomka cultivar showed the lowest reduction of 5.18% at the flowering stage under stress conditions compared to the Master (8.45%), Azargol (7.33%), and Euroflor (9.8%). In the results obtained from biological performance, the Azargol cultivar showed the lowest reduction in all three stages of head formation, flowering, and grain filling, respectively, by 3.05%, 18.19%, and 4.07%, compared to the Master cultivar (22.84%), (26.95%), and (20.16%).

Keywords: sunflower, stress, yield, azargol

Introduction

Environmental stresses are considered one of the most important factors reducing the yield and production of crops, and combating or reducing the effects of stresses has been considered as a useful strategy to increase the yield of these crops. Drought stress is one of the major challenges for the successful production of crops, and in this regard, breeding advanced and tolerant varieties for arid and semi-arid regions seems essential. Sunflower is one of the main sources of vegetable oil in the world and Iran. Water deficit during the growing season of sunflower can reduce net photosynthesis by reducing leaf area, closing stomata, and reducing the efficiency of carbon dioxide absorption. Water deficit stress during the growing season reduces the dry weight of leaves, stems, and roots, and also increases the root to aerial ratio due to deeper roots. Despite relative drought tolerance, water shortage is one of the most important factors limiting sunflower cultivation in Iran and the world. expanding the area under cultivation and increasing the production of sunflowers requires the introduction of cultivars adapted to dry and semi-arid conditions (Zareei Siahbidi et al, 2020). Sunflower hybrid varieties have very desirable characteristics in terms of uniformity, disease resistance, and yield. to their genetic homogeneity, they are very sensitive to changes in the growing environment. In contrast, open-pollinated varieties are less sensitive to changes in environmental conditions due to their heterogeneous genetic makeup and show good adaptability in different conditions. In addition, open-pollinated varieties are a very suitable option for cultivation

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in dryland areas and areas with agricultural restrictions. In all cross-pollinated plants, hybrid varieties have high yields due to the heterosis phenomenon, and single-cross hybrids are more performance compared to other types of hybrids. Commercial varieties are low yielding and uneven, such as Record, Armavirskiy and Chernyanka. Hybrid varieties such as Azargol, Eurflor, lakomka and Master are high yielding and more uniform (Ghaffari et al., 2021). In this research, Euroflor and Azargol cultivars were considered as single-cross hybrids, and Lakomka and Master cultivars were considered as open-pollinated cultivars. The Azargol hybrid was of Iranian origin and the rest were Iranian-Russian. All four cultivars had a growth period of 110 to 120 days and were resistant to plasmospora disease. This plant has received increasing attention over the past few decades due to its beneficial fatty acids. Evaluation of various characteristics, especially the performance of genotypes under normal and moisture stress conditions, allows the identification of tolerant genotypes (Tarig et al., 2021). introducing tolerant genotypes into breeding programs, an effective step can be taken to reduce the adverse effects of drought stress and human food security (Gholamhoseini et al., 2013). In order to evaluate and identify tolerant and sensitive genotypes, several methods have been proposed by researchers in this field, one of which is the use of stress tolerance and sensitivity indices (Nawar et al, 2020; Darbani et al, 2020). In this Research, given that the cultivars considered for cultivation had acceptable yields, it was decided to examine these cultivars under stress conditions and at different phenological stages to see which cultivars gave acceptable yields at different times of stress.

MATERIALS AND METHODS

In order to evaluate sunflower cultivars under the influence of water shortage stress, an experiment was conducted on four sunflower cultivars in the form of a split plot in the form of a basic randomized complete block design in 3 replications at the research farm of Khoy Agricultural Research Station in 2024. Stress treatment was carried out at four levels including: no water stress, stress during tillering, stress at the flowering stage, stress at the seed filling stage in the main plots and cultivars at four levels including Master, Lakomka and Euroflor and Azargol hybrids in the secondary plots. The seeds were obtained from the Karaj Seedling and Seed Institute, and the goal was to breed these varieties under stress conditions and adapt to the Azerbaijani climate. The project implementation site has a semi-arid climate and is located at 58˚44´ north latitude and 33˚48´ east latitude, and its altitude is 1142m above sea level. Planting was done on land that had been fallow the previous year. Bed preparation operations included plowing, spring discing, and leveling. A period of 120 days passed from planting to harvest. In order to determine some physical and chemical characteristics related to the soil of the test site before implementing the project, a sample of the soil composite was prepared from an average depth of 30 cm and sent to the soil science laboratory of the Soil and Water Research Department of the Agriculture and Natural Resources Research Center of West Azerbaijan (Table 1).

Simultaneously with the land preparation operation, 150 $kg.ha^{-1}$ of diammonium phosphate fertilizer and 100 kg of potassium sulfate were applied to the land. Also, 100 $kg.ha^{-1}$ of urea fertilizer was evenly distributed in three stages: at planting, at the 5-leaf stage, and at the beginning of flowering in the experimental units. In this experiment, each sub-plot had 4 planting lines at 60 cm intervals, 5 m long, and an area of 12 m^2. After planting, initial irrigation was performed every 5 days until the plants were established and then until the end of the season based on 80 mm of evaporation from the class A evaporation pan. In stress treatments, depending on the stress stage, irrigation was performed when the evaporation rate from the class A evaporation pan reached 110 mm, and subsequent irrigations were performed according to normal conditions (irrigation after 80 mm from the class A evaporation pan). Stress treatments were applied at four levels including: no water stress, stress at pod formation, stress at flowering stage, stress at seed filling stage and irrigation time including irrigation at 80 (control), 100, 110 and 120 mm evaporation from class A pan as main factors. Also, Master, Lakomka and Euroflor and Azergol hybrids were placed in subplots. At harvest time, traits such as number of grain per head, head diameter, 1000 grain weight, biological yield, harvest index, and sunflower seed yield were measured as follows. For thousand seed weight measurement, three 100-seed samples from each plot were counted and weighed after drying in an oven. Finally, the average weight obtained was multiplied by 10 and recorded as thousand seed weight. For biological yield, the whole plant was placed in an oven at

Table 1. Physico-chemical properties of soil experimental location

sand	Silt	Clay	pH	Soil saturation	P available (ppm)	K available (ppm)	Fe avalable (ppm)	Total N	OC
18	36	46	7.8	46	9.4	361	4.9	0.08	0.87

75°C for 48 hours and weighed. Then, by taking a sample of the obtained seeds, one thousand seeds were counted and then the weight of 1000 grain weight was measured using a precision balance (accuracy 0.01 g). To calculate the harvest index, the number of seven harvested samples after separating the seeds from the pod and drying in an oven (48 hours at 72°C) was weighed and the following formula was used: Harvest index = yield / (biological yield) × 100

Statistical analysis

The resulting data were statistically analyzed with Minitab 14 software and the averages of main and interaction effects were compared with the LSD test at the five percent probability level.

Results and Discussion

Grain yield

The results of the analysis of variance of the data showed that there was a significant difference in grain yield at the level ($P \leq 0.01$) between different levels of stress as the main plot, cultivars in the subplot and their interaction effects (Table 2). Azargol variety, in the presence of stress in head formation, reduces its yield by 9.68%, while the Euroflor variety shows a 18.24% decrease. Alsot the Azargol cultivar, in the presence of flowering stress, reduces its yield by 28.57%, while the Euroflor cultivar shows a reduction of 37.17%. Finally, the Azargol cultivar reduces its yield by 18.86% in the presence of grain filling stress, while the Lakumka cultivar showed a 25.64% reduction. Also, the results of the interaction effects showed that the Azargol cultivar had the highest yield in non-stressed conditions with 3084 kg, and the Master cultivar had the lowest grain yield in the grain formation stage with 2654 kg (Table 4). Azargol cultivar has better yield than other cultivars under stress and non-stress conditions, and also stress at the grain formation stage until the end of the growth period, considering that the plant is exposed to drought stress for a longer period, has the greatest effect on yield components. Duca & Domenco (2022) also reported that the occurrence of severe water deficit stress during flowering, pollination, and grain setting stages causes a severe reduction in sunflower seed yield. Jocković et al. (2024) stated that an unfavorable irrigation regime, while reducing the surface area of leaves and their premature aging, causes a decrease in seed yield.

Harvest index

The results showed that the harvest index was also affected by stress × cultivar. (Table 2), in the presence of stress, the Master cultivar showed the lowest reduction in harvest index at the stage of head formation and grain filling (7.08% and 5.25%, respectively) compared to the Euroflor cultivar (10.94%) and (8.31%), and the Lakomka cultivar showed the lowest reduction of 5.18% at the flowering stage under stress conditions compared to the Master (8.45%), Azargol (7.33%), and Euroflor (9.8%). The highest harvest index in non-stress conditions was for the Euroflor variety with 25.27% at the grain filling stage and the lowest was for the Lakumka variety with 23.16% at the grain filling stage (Table 4). Ali et al. (2024) reported that due to the decrease in grain yield and biological yield due to low irrigation, the yield index of hybrids increased, so that under mild stress conditions, the yield index of the Farrokh, Shams, Ghasem and Barzegar hybrids increased by 18, 4, 15 and 3 %, respectively, and this increase under severe stress conditions was equal to 5, 21, 5 and 7 %, respectively. Increasing harvest index in the studied hybrids under both limited irrigation conditions resulted in a more severe decrease in biological yield compared to grain yield. The results of Sandoval et al., (2024) also showed that mild drought stress increases the harvest index. In the study of Saylak et al. (2024), no significant changes in the harvest index of the studied genotypes were observed due to drought stress. Giannini et al. (2024) reported that the reduction in harvest index was not large compared to the reduction in grain yield, indicating that the rate of grain yield reduction was only slightly higher than the rate of plant dry matter reduction. However, in several other studies, drought stress caused a significant reduction in harvest index (Masumi et al., 2021; Umar & Siddiqui., 2020). The reduction in harvest index resulted in a decrease in the number of grains per head, a decrease in head diameter, and an increase in grain porosity (Hussein et al., 2018). This indicates that drought stress leads to a decrease in the biological yield of plants and an increase in the harvest index of the treatments, which is consistent with the results of this study.

Biological yield

Biological yield showed significant differences between different levels of stress, cultivar and their interactions at the ($P \leq 0.01$) (Table 1). In the results obtained from biological performance, the Azargol cultivar showed the lowest reduction in all three stages of head formation, flowering, and grain filling, respectively, by 3.05%, 18.19%, and 4.07%, compared to the Master cultivar (22.84%), (26.95%), and (20.16%), Also, the Azargol cultivar had the highest biological yield in non-stressed conditions with 15,500 kg, and the Master cultivar had the lowest biological yield in the seed formation stage with 12,350 kg (Tables 2 and 4). In the experiment (Joseph et al, 2025), it was found that mild and severe drought stress reduced the biological yield of sunflower by 43 and 69%, respectively. The reduction in biological yield due to drought stress can be due to the reduction in leaf area and the reduction in plant growth rate (Mendes et al., 2025; Avetisyan & Cvetanova (2022). In the study of Önemli et al. (2023), biological yield also decreased under stress conditions at the flowering stage, and this decrease caused a decrease in leaf area index and crop growth rate. They also reported that the rate of decrease in dry matter in plants under drought stress was greater than the rate of decrease in grain yield.

1000 grain weight

The 1000 grain weight for different stress levels and cultivars was significant at the ($P \leq 0.01$) (Table 2) Azargol variety, in the presence of stress in head formation, reduces its weight 1000 grain by 10.14%, while the Euroflor variety shows a 17.03% decrease. Alsot the Master cultivar, in the presence of flowering stress, reduces its yield by 9.18%, while the lakomka cultivar shows a reduction of 21.02%. Finally, the Azargol cultivar reduces its weight 1000 grain by 5.7% in the presence of grain filling stress, while the master cultivar showed 9.28% reduction., Also, the Azargol cultivar had the highest 1000 grain weight in non-stressed conditions with 78.65gr, and the Master cultivar had the lowest 1000 grain weight in the filling formation stage with 76.73 gr (Table 3). Centorame et al., (2024) showed that the effect of drought stress on the physical characteristics of sunflower seeds, including length, width, and diameter, was negative, and the reason for this was due to the reduction in the production of photosynthetic materials required for seed filling, which ultimately caused the seeds to shrink and reduce the 1000 grain weight. In the experiment (Smaeili et al., 2022; Tariq et al., 2021), it was reported that by stopping irrigation from the flowering stage in sunflower, the number of seeds per plant decreased and the competition for photosynthetic materials decreased, and the weight of seeds increased. It seems that moisture stress, through a decrease in leaf area, caused a decrease in seed weight and the number of seeds per plant. The competition between fertile florets and seeds on the surface for the absorption of photosynthetic materials was not intense in the Lakomka cultivar, which ultimately led to an increase in the seed weight of this cultivar.

The number of grain in head

Given that there was no significant difference in the number of grains between different stress levels, it seems that the 1000 grain weight is the most effective factor in the difference in performance at different stress levels. For the number of grains per head, there was no significant difference between different levels of moisture stress and the interaction effects of stress × cultivar However, cultivars had a significant effect on this trait at the ($P \leq 0.01$) .(Table 2), Euroflor variety, in the presence of stress in head formation, reduces its The number of grain in head by 5.16%, while the Lakomka variety shows a 18.45% decrease. Also the Azargol cultivar, in the presence of flowering stress, reduces its The number of grain in head by 7.46%, while the lakomka cultivar shows a reduction of 21.59%. Finally, the Azargol cultivar reduces its The number of grain in head by 9.4% in the presence of grain filling stress, while the master cultivar showed 24.68% reduction, Also, the Euroflor cultivar had the highest The number of grain in head in non-stressed conditions with 794, and the Lakomka cultivar had the lowest The number of grain in head in the filling formation stage with 620.

Head diameter

There was a highly significant difference between different levels of stress and cultivars in terms of head diameter ($P \leq 0.01$). Azargol variety, in the presence of stress in head formation, reduces its head diameter by 19.95%, while the Master variety shows a 29.55% decrease. Also the Azargol cultivar, in the presence of flowering stress, reduces its head diameter by 11.26%, while the lakomka cultivar shows a reduction of 25.10%. Finally, the Azargol cultivar reduces its head diameter by 17.19% in the presence of grain filling stress, while the Master cultivar showed 24.27% reduction, but their intersept effects did not show a significant difference (Table 2). Also, the Lakomka cultivar had the highest head diameter in non-stressed conditions with 22.8cm, and the Euroflor cultivar had the lowest head diameter in the filling formation stage with 20.2cm. (Table 2).(Akhtar et al., 2024; Sah et al., 2020) showed that the maximum stem diameter of 17.2 cm and the maximum yield of 5124 $kg.ha^{-1}$ belonged to the treatment of 70-70-70 mm evaporation from the surface of the pan, class A evaporation, respectively, from the stage of plant establishment to the appearance of the head, to physiological maturity., and the two cultivars of Record and High Sun 33 showed better grain yield. The occurrence of drought stress during the vegetative period causes a decrease in the number of leaves and finally the leaf surface, and as a result, the total photosynthetic materials produced for proper growth and high yield are reduced. The continuation of the low water stress process in the stage of emergence until pollination causes a decrease in the diameter of the stalk due to the decrease in the transfer of photosynthetic materials, and finally, the occurrence of drought stress in the reproductive stage can have a significant effect on the head diameter. Other tests have also been reported by (Tariq et

Table 2. Results of analysis of variance of some traits affected by drought stress in different sunflower cultivars

s.o.v	df	Grain yield	biological yield	harvest index	1000 grain weight	Head diameter	No. of grain in head
Replication	2	0.421^{ns}	60.80	43.813	1.071	0.813	9162.25
Stress levels A	3	13.461**	323.624**	30.996*	317.17**	156.08**	122592.7^{ns}
Error A	6	1.346	28.073	7.085	3.217	11.951	112602.9
Cultivars	3	1.334**	52.224**	29.897**	418.565**	4.46*	76399.2**
Cultivars*stress	9	0.646**	4.673**	12.803**	6.202^{ns}	2.65^{ns}	10648.2^{ns}
Error B	24	0.206	1.384	4.425	2.745	1.167	4791.93
CV %		14.97	9.22	10.28	2.79	5.98	9.72

*and** are significant at the five and one percent probability levels, respectively

Table 3. Comparison of the mean of some traits affected by drought stress (main plot) in different cultivars (subplot)

Experimental factors	Head diameter	1000grain weight	harvest index	biological yield	Grain yield	No.of grain in head
Non stress	23a	78.85a	15.46b	19946 a	3084 a	814a
Stress stage formation head	14.3c	64.03d	17.09ab	7106 c	1215 c	586.7a
Stress in the flowering stage	17.5b	69.25c	17.33a	12222 b	2119 b	769a
Grain filling stage stress	17.4b	71.35b	18.22a	12696b	2314b	677.6a
master	17.7 a	66.7c	17.72ab	10536b	1867b	671.9b
Lakomka	17.6 a	80.24a	17.47a	12523ab	2189a	620.7b
Azargol	18.9a	72.20b	13.15c	15667a	2061a	760.6a
Euroflor	18.1 a	64.4d	14.27bc	13243ab	1891a	794.5a

al, 2018). Head diameter is one of the most basic traits that decreases under the influence of moisture stress and has a negative effect on yield components such as the number of seeds per head (Khoufi et al, 2013) observed that drought stress always has a negative effect on head diameter and one of the goals Sunflower breeding is the selection of genotypes with a larger diameter. The results (Janzen et al., 2023) showed that the rate of decrease of dry matter in the plant due to drought stress was higher than the rate of decrease of grain yield. In the study of Abassi et al. (2021) it has also been pointed out that the weight of aerial organs is severely reduced due to stress. Kaya et al.(2016) reported a 22-50% reduction in the weight of aerial organs due to drought stress. Sometimes the results presented in this regard are different,

Table 4. Comparison of the average interaction effects of some traits affected by drought stress in different cultivars

Experimental factors	biological yield	Grain yield	harvest index
non Stress			
Master	16651c	2688bc	def 16.14
Lakomka	19354b	3096 b	def 15.99
Azargol	24624a	3732 a	ef 15.15
Euroflor	19150b	2736 bc	f 14.28
Stress stage formation head			
Master	6154i	1032h	16.76cdef
Lakomka	7199ih	1500gh	a 20.83
Azargol	8372ch	1308gh	ef 15.62
Euroflor	6698hi	1014h	ef 15.13
Stress in the flowering stage			
Master	9860fg	1872ef	18.98abc
Lakomka	10769f	1740 efg	16.15cdef
Azargol	14926c	2580 bcd	17.28 bcde
Euroflor	13336de	2262 cde	16.96 bcde
Grain filling stage stress			
Master	9477fg	1878ef	19.81ab
Lakomka	12733e	2040 bcd	16.02a
Azargol	14747d	2142 de	14.52ef
Euroflor	13787de	2580 bcd	18.71 abcd

so that in the study of Mohammadi Alagoz et al., (2023), the decrease in grain yield was greater than the decrease in biological yield (Ahmed et al., 2024).

Averages with common letters in each column have no significant difference at the five percent probability level with Duncan's test

Conclusions

Given that most countries, including Iran, will face water crises in the future, genetic improvement and introduction of drought-resistant cultivars are essential. In this study, hybrid and open-pollinated sunflower cultivars were evaluated under drought stress conditions and at different phenological stages, and among them, the Azargol cultivar is introduced as the superior cultivar due to the least reduction in grain yield under water deficit conditions, which is probably due to the less reduction in photosynthesis, enzymatic activity, and re-transfer of storage materials from other organs to seeds, and as a result, less reduction in the weight of filled seeds.

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References

Abassi, B., Mohammadi, G., & Bagheri, A. (2021). Study the effect of living mulch on phenological characteristics and quantitative and qualitative yield of sunflower cultivar. *Journal of Agricultural Science and Sustainable Production, 31(3*), 51-69.

Aghdam, M. Z., Kojouri, F. D., Ghaffari, M., & Ebrahimi, A. (2019). Genetic Analysis of Morpho-Physiological Characteristics of Sunflower Under Stress and Non-Stress Drought Conditions. *Agrivita: Journal of Agricultural Science*, *41*(3).

Ahmed, M., Ahmad, S., Abbas, G., Hussain, S. & Hoogenboom, G. (2024). Sunflower-Sunflower System. In: *Cropping Systems Modeling Under Changing Climate* (pp. 109-141). Singapore: Springer Nature Singapore.

Akhtar, N., Yousaf, M. A., Shaukat, S., Gul, S., Saleem, U., Mahmood, T., ... & Asif, M. (2024). APPRAISAL OF SUNFLOWER (HELIANTHUS ANNUUS L.) HYBRIDS FOR MORPHOPHENOLOGICAL TRAITS UNDER NORMAL AND TERMINAL HEAT STRESS CONDITIONS. *SABRAO Journal of Breeding & Genetics*, *56*(6).

Ali, Q., Ali, M., Khan, F. Z. A., Noureldeen, A., Alghamdi, A., Darwish, H., ... & Saeed, S. (2024). Water Deprivation and Sowing Times Alter Plant–Pollination Interactions and Seed Yield in Sunflower, Helianthus annuus L.(Asteraceae). *Plants, 13(*22), 3194.

Avetisyan, D., & Cvetanova, G. (2022). Assessment of drought impact on phenological development of selected sunflower hybrids based on vegetation indices and orthogonalization of multispectral satellite data. *Bulgarian Journal of Agricultural Science*, *28*(6).

Centorame, L., Ilari, A., Del Gatto, A., & Pedretti, E. F. (2024). A systematic review on precision agriculture applied to sunflowers, the role of hyperspectral imaging. *Computers and Electronics in Agriculture*, *222*, 109097.

Darbani, S. P., Mehrabi, A. A., Pordad, S. S., Maleki, A., & Farshadfar, M. (2020). Effect of drought stress on agro-morphological traits in sunflower (Helianthus annuus L.) genotypes and identification of informative ISSR markers. *Plant Genetic Resources*, *18*(2), 49-62.

Duca, M., Boian, I., & Domenco, R. (2022). The impact of droughts on sunflower production in the Republic of Moldova. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 1-16.

Ghaffari, M., Gholizadeh, A., Andarkhor, S. A., Zareei Siahbidi, A., Kalantar Ahmadi, S. A., Shariati, F., & Rezaeizad, A. (2021). Stability and genotype× environment analysis of oil yield of sunflower single cross hybrids in diverse environments of Iran. *Euphytica*, *217*(10), 187.

Gholamhoseini, M., Ghalavand, A., Dolatabadian, A., Jamshidi, E., & Khodaei-Joghan, A. (2013). Effects of arbuscular mycorrhizal inoculation on growth, yield, nutrient uptake and irrigation water productivity of sunflowers grown under drought stress. *Agricultural Water Management*, *117*, 106-114.

Giannini, V., Mula, L., Carta, M., Patteri, G., & Roggero, P. P. (2022). Interplay of irrigation strategies and sowing dates on sunflower yield in semi-arid Mediterranean areas. *Agricultural Water Management*, *260*, 107287.

Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M., & Nawaz, A. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agricultural water management*, *201*, 152-166.

Janzen, G. M., Dittmar, E. L., Langlade, N. B., Blanchet, N., Donovan, L. A., Temme, A. A., & Burke, J. M. (2023). Similar transcriptomic responses to early and late drought stresses produce divergent phenotypes in sunflower (Helianthus annuus L.). *International Journal of Molecular Sciences*, *24*(11), 9351.

Jocković, M., Jocić, S., Cvejić, S., Dedić, B., Jocković, J., Ćuk, N., ... & Miladinović, D. (2024). Unveiling sunflower morphological and phenological adaptations to drought stress. *Oilseeds and fats, Crops and Lipids (OCL)*, *31*, 29.

Joseph, C. O., Kambwily, K., & Emmanuel, S. M. (2025). Effects of planting window on grain yield and oil yield under rainfed sunflower (Helianthus annuus L.) in Kilwa District, Lindi, Tanzania. *Discover Agriculture*, *3*(1), 8.

Kaya, Y., Nalcaiyı, A. S. B., Erdal, S. C., Arslan, O., Cıcek, N., Pekcan, V., ... & Ekmekcı, Y. (2016). Evaluation of male inbred lines of sunflower (Helianthus annuus L.) for resistance to drought via chlorophyll fluorescence. *Turkish Journal of Field Crops*, *21*(2), 162-173.

Khoufi, S., Khamassi, K., Teixeira da Silva, J. A., Aoun, N., Rezgui, S., & Ben Jeddi, F. (2013). Assessment of diversity of phenologically and morphologically related traits among adapted populations of sunflower (Helianthus annuus L.). *Helia*, *36*(58), 29-40.

Masumi, M., Moradi, R., Pourghasemian, N., & Naghizade, M. (2021). Assessing the Comparative Advantage of Naked-Seeded Pumpkin (Cucurbita pepo L.) and Sunflower

(Helianthus annuus L.) Intercropping as affected by Drought Stress. *Journal of Agroecology*, *13*(4), 631-652.

Mendes, C. R. L. G., de Sousa Ferreira, L., Carnietto, M. R. A., Almeida, L. C. O., da Silva, G. F., Santos, H. L., ... & de Almeida Silva, M. (2025). Gibberellin biosynthesis inhibitors distinctly affect sunflower morphometric and growth dynamics. *South African Journal of Botany*, *179*, 98-111.

Mohammadi Alagoz, S., Hadi, H., Toorchi, M., Pawłowski, T. A., Asgari Lajayer, B., Price, G. W., ... & Astatkie, T. (2023). Morpho-physiological responses and growth indices of triticale to drought and salt stresses. *Scientific Reports*, *13*(1), 8896.

Nawar, A. I., Salama, H. S., & Khalil, H. E. (2020). Additive intercropping of sunflower and soybean to improve yield and land use efficiency: Effect of thinning interval and nitrogen fertilization. *Chilean journal of agricultural research*, *80*(2), 142-152.

Önemli, F., & Önemli, G. (2023). Evaluation of Wild Annual Sunflower Species for Some Morphological, Phenological, and Agronomic Characters under Field Conditions. *Tekirdağ Ziraat Fakültesi Dergisi*, *20*(4), 857-870.

Sah, R. P., Chakraborty, M., Prasad, K., Pandit, M., Tudu, V. K., Chakravarty, M. K., ... & Moharana, D. (2020). Impact of water deficit stress in maize: Phenology and yield components. *Scientific reports*, *10*(1), 2944.

Sandoval, M. N., Cirilo, A. G., Paytas, M. J., Zuil, S. G., & Izquierdo, N. G. (2024). Critical periods for waterlogging effects on yield and grain components in sunflower (Helianthus annuus), soybean (Glycine max) and sorghum (Sorghum bicolor): a comparative study. Journal of Agronomy and Crop Science, 210(5), e12765.

Saylak, S., Irmak, S., Eskridge, K. M., & Dweikat, I. (2025). Sunflower germplasms' response to different water and salinity stress levels in greenhouse and field conditions under subsurface drip irrigation. *Irrigation and Drainage*, *74*(1), 161-181.

Smaeili, M., Madani, H., Nassiri, B. M., Sajedi, N. A., & Chavoshi, S. (2022). Study of water deficiency levels on ecophysiological characteristics of sunflower cultivars in Isfahan, Iran. *Applied Water Science*, *12*(5), 108.

Tariq, M., Ahmad, S., Fahad, S., Abbas, G., Hussain, S., Fatima, Z., ... & Hoogenboom, G. (2018). The impact of climate warming and crop management on phenology of sunflower-based cropping systems in Punjab, Pakistan. *Agricultural and Forest Meteorology*, *256*, 270-282.

Tariq, M., Fatima, Z., Iqbal, P., Nahar, K., Ahmad, S., & Hasanuzzaman, M. (2021). Sowing dates and cultivars mediated changes in phenology and yield traits of cotton-sunflower cropping system in the arid environment. *International Journal of Plant Production*, *15*(2), 291-302.

Umar, M., & Siddiqui, Z. S. (2020). Florescence assessment of sunflower genotypes against drought stress environment. *Pak. J. Bot*, *52*(4), 1181-1188.

Zareei Siahbidi, A., Rezaizad, A., & Daneshian, J. (2020). Effect of deficit irrigation on seed yield and yield components of sunflower (Helianthus annuus L.) hybrids. *Iranian Journal of Crop Sciences*, *22*(1), 1-14.

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Pignatti, S. (1982). Reprint 1997. Flora d'Italia. Edagricole, Bologna (IT), 3Vols.

Barbour, M. G., & Billings, W. D. (Eds.). (2000). North American terrestrial vegetation. Cambridge University Press.

Bennett, K.D., (2005). Documentation for psimpoll 4.25 and pscomb 1.03: C programs for plotting pollen diagrams and analysing pollen data. Retrieved from http://chrono.qub.ac.uk/psimpoll/psimpoll.htmlTitles of journals should be given in full.

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