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The effect of different growing locations on the morphological characteristics and flavonoid content of clove plants (*Syzygium aromaticum*)

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ABSTRACT

This study investigated the effects of growing location factors on the morphological characteristics and secondary metabolite content (flavonoid) of clove. It was conducted in three locations, namely Nganjuk, Jombang, and Trenggalek, from November 2023 to February 2024. The study began with an agroclimatological analysis, including air temperature, air humidity, light intensity, and soil pH. Subsequently, the morphological characteristics of clove plants were analyzed, and clove leaf samples were collected at the three locations to test their flavonoid content. The flavonoid analysis was performed at the CNH Laboratory in Semarang, Central Java, using the UV-Vis Spectrophotometry method. The results indicated that differences in growing locations and environmental conditions affected the morphological characteristics of clove plants. The morphological traits that showed variation among the three locations included stem surface, stem color, leaf shape, leaf apex, leaf color, and leaf size. Additionally, the environmental differences influenced the flavonoid content in clove leaves, with the highest flavonoid content (0.049%) observed in leaves from Jombang. The practical implication of this study is that selecting cultivation sites for clove should consider agroclimatic conditions and soil type. The observed morphological differences among locations, despite being of the same variety, reinforce the understanding that plant adaptation to the environment results from interactions between genetic and environmental factors.

Contribution/Originality: This study documents the simultaneous examination of the effects of growing location on both morphological traits and leaf flavonoid content of clove in East Java. By integrating agroclimatological analysis, the research reveals that environmental variability significantly influences phenotypic expression and secondary metabolite accumulation, even within a single clove variety.

1. INTRODUCTION

Clove (*Syzygium aromaticum*), a member of the *Myrtaceae*, is a spice plant that has many benefits, including as a culinary spice, herbal medicine, and an ingredient in fragrance. The leaves, flowers, and stalks of cloves can be used as raw materials for industrial needs (Elisa Loppies, Wahyudi, Sri Rejeki, & Aulia Winaldi, 2021). Clove plants have several varieties, one of which is the Zanzibar variety. According to Marboen (2019), the Zanzibar variety has many advantages, one of which is that each fruit stalk contains hundreds of seeds and bears fruit more often than other types of cloves. In addition, the size of Zanzibar cloves is larger than other varieties. Clove oil from the Zanzibar variety often has a more intense and unique aroma and flavor, making it the first choice in the perfume, aromatherapy and food flavoring industries (Khamis, Suleiman, Sheikh, & Ali, 2021).

Different growing locations can indicate different soil types in those locations. The soil type affects the texture, structure, and nutrient content in the soil. According to Kumari et al. (2022) soil type is important for growth because lack of nutrients from the soil can cause stress on plants (abiotic stress). Different growing locations of a plant can affect its morphological characteristics (Widiya, Jayati, & Fitriani, 2019). Morphology is a branch of science in Biology that studies the physical structure or body of plants (Mayoru, Jufri, & Usman, 2022). Morphological differences can occur due to differences in environmental conditions thus, the plants must adapt to the existing conditions (Hadiyanti, Supriyadi, & Pardono, 2018). The altitude level is related to the intensity of light, which is an ecological resource providing photosynthesis and directly affects the survival of plant growth. Keleş (2020) stated that morphologically, plants can adapt to different elevation gradients by changing the height and diameter of the stems, the length of the segments, and the thickness of the bark.

Moreover, different growing locations also affect the level of secondary metabolites in plants (Kopon, Baunsele, & Boelan, 2020). Several studies, including studies conducted by Manurung, Hidayati, Wijayanti, and Nuringtyas (2021); Rahmawati, Santosa, and Purwanto (2023); Katuuk, Wanget, and Tumewu (2019) and Setyo Utomo, Kristiani, and Mahardika (2020) stated that location is one of the factors that affect metabolism a few seconds later. The flavonoid content in plants will vary by region due to environmental factors, including rainfall intensity, soil type, and growth rate, all of which play a role in determining plant nutrition (Lallo et al., 2020).

Clove leaves contain secondary metabolites that play a role in attracting other organisms, protecting against pathogenic agents, protecting and adapting to environmental stress, protecting against ultraviolet light, serving as a growth control substance, as well as facilitating competition with other plants (allelopathy) (Dalimunthe & Rachmawan, 2017). One of the metabolic products contained in clove leaves is flavonoid (Wahyulianingsih, Handayani, & Malik, 2016). It has a role in the physiological functions of plants and shows the ability to protect plants from stress originating from living things (biotic) and the environment (abiotic) (Shah & Smith, 2020). Flavonoid production in plants can be influenced by various environmental stresses, such as the availability of nutrients like nitrogen and phosphorus in the soil (Shah & Smith, 2020).

The amount of flavonoid compounds will increase under light exposure. Light affects the properties of polysaccharides and phenolic synthesis and can increase antioxidant activity (Atif, Amin, Ghani, Ali, & Cheng, 2020). Flavonoid compounds have toxic/ allelopathic properties and have a very strong aroma, bitter taste, dissolve in polar solvents and easily decompose at high temperatures (Talahatu & Papilaya, 2015). Based on this background, the researchers identified the effects of different growing locations on the morphological characteristics and flavonoid content of clove plants. This study can serve as a reference for superior clove cultivation.

2. METHODS

This study was conducted from November 2023 to February 2024 at three different clove plant locations. Location 1 was in *Sawahan* (*Nganjuk*) with an altitude of 614 m above sea level and had Andosol soil type (BPS Nganjuk, 2021). Location 2 was in *Wonosalam* (*Jombang*) with an altitude of 525 m above sea level and had black alluvial soil type (Sabdopalon-Jombang Regency Government, 2023). The last location 3 was in *Kampak* (*Trenggalek*) with an altitude of 200 m above sea level and had Mediterranean Mix soil type (BPS Trenggalek Regency, 2020). The tools used in the morphological characteristics' measurements were stationery, pH meter, thermometer, hygrometer, lux meter, coordinate maps, plastic ruler, soil tester, digital scale, and camera, while the materials used in the total flavonoid content test were clove leaves from three locations: *Jombang*, *Nganjuk*, and *Trenggalek*. The methods used in this study were qualitative descriptive, location survey, sampling, and plant morphology observation. The sampling was conducted using purposive random sampling. In analyzing the flavonoid content of clove leaves, the UV-Vis spectrophotometry method was used, and for the morphological characteristics, they were analyzed using Principal Component Analysis (PCA) with PAST 4.0.

3. RESULTS

3.1. Results of Agroclimatology Analysis

Based on the results of the environmental parameter observations at the three research locations, there were significant differences between Nganjuk and Jombang in terms of agroclimatology observations. Table 1 presents the results of abiotic environmental observations of clove plants at three locations: Nganjuk, Jombang, and Trenggalek. The air temperature in Jombang is higher than in Nganjuk, while the air humidity tends to be higher in Trenggalek. Meanwhile, the light intensity and soil pH in Jombang are higher than at the other locations. The interpretation of these differences can be a determining factor in the variation of clove plants' morphological characteristics in each location. Differences in temperature and light intensity can affect plant growth and development. Meanwhile, differences in air humidity and soil pH can influence the availability of water and nutrients for the plants. Therefore, considering environmental factors is important in evaluating the adaptation of clove plants to different environments and the potential variations in plant characteristics at each location. Jombang has a slightly higher temperature (29.3°C), which remains within the ideal range; however, caution is warranted if field temperatures rise toward or above 35°C, as cloves can experience heat stress at excessive temperatures, manifested as wilting and flower drop. In contrast, Trenggalek has a slightly cooler temperature (27.35°C), which poses no hindrance to growth. In fact, lower temperatures, especially at night, can reduce respiration, allowing the plant to conserve energy for growth. Moreover, all observed temperatures are well above the critical minimum (10°C), below which cloves suffer cold stress.

Table 1. Results of agroclimatology analysis in three locations.

Observation parameters	Information		
	Nganjuk (614 m asl)	Jombang (525 m asl)	Trenggalek (200 m asl)
Air temperature (°C)	28.3	29.3	27.35
Air humidity (%)	71%	62%	84%
Light intensity (Lux)	535.5	1.532	976.75
Soil pH	6.6	6.8	5.8
Soil type	Andosol	Black Alluvial	Mediterranean mix

Source: Primary data of the study (2023).

Relative humidity plays a crucial role in processes like transpiration and nutrient uptake. Clove trees generally thrive in high-humidity environments. The 84% relative humidity in Trenggalek approaches an ideal level (around 80% or higher) for cloves, allowing stomata to remain open longer without excessive water loss. This supports optimal photosynthesis and vegetative growth. However, very high humidity combined with warm temperatures can increase disease risk, particularly fungal infections on clove trees. In Jombang, with a lower humidity (62%), clove plants may experience higher transpiration rates. The relatively low light level in Nganjuk may have been due to cloud cover or shading (given Nganjuk is higher elevation, which might see more clouds).

Differences in light intensity among the sites also affect clove growth. As a tropical tree, cloves require abundant sunlight (full sun) for optimal productivity, although young plants prefer partial shade. Jombang's high light intensity likely provides more energy for photosynthesis. If water and nutrients are ample, this can enhance growth and reproductive development (flowering and fruiting).

In terms of soil properties, the three locations have soil pH ranging from slightly acidic to neutral. Trenggalek's soil was the most acidic (pH 5.8), whereas Nganjuk (pH 6.6) and Jombang (pH 6.8) were near neutral. The soil types also differed: Nganjuk's soil is andosol (young volcanic soil, typically fertile with a sandy loam texture), Jombang's is black alluvial soil (organically rich river deposit), and Trenggalek's is a Mediterranean mix soil (generally a reddish-brown calcareous soil with fast drainage). These differences imply variations in drainage and fertility, for example, andosols are known for high organic matter content and good water retention, whereas Mediterranean-type soil tends to be calcareous with rapid drainage, potentially making it drier.

Differences in soil pH and type across the three locations influence nutrient availability and root development. Soils in Ngnjuk (Andosol, pH 6.6) and Jombang (alluvial, pH 6.8) are near neutral, a range that maximizes the availability of macronutrients (N, P, K) for plants. Trenggalek's soil (pH 5.8) is moderately acidic; at this pH, certain nutrients (e.g., phosphorus) become less available. However, cloves are fairly tolerant of mildly acidic to neutral soils, so a pH of 5.8 is still within a tolerable range, especially if the soil has sufficient organic matter.

Nganjuk's andosol soil, derived from volcanic ash, is typically rich in minerals and organic matter and has good porosity, supporting robust root growth and moisture retention. Jombang's black alluvial soil is very fertile due to high organic content and river-derived mineral deposits, providing plentiful nutrients for clove growth. Trenggalek's Mediterranean mix soil likely drains quickly and may contain less organic material; consequently, clove roots there may need to grow deeper to access water, especially during dry periods.

Trenggalek at 200 m represents clove's original lowland habitat (wet tropical elevation). At mid elevations, temperatures are slightly cooler and dry seasons are more pronounced; these conditions can induce clove flowering as long as drought stress is kept under control. In contrast, a continuously humid lowland environment like Trenggalek promotes lush vegetative growth but may require a brief dry spell to trigger optimal flowering.

3.2. Morphological Characteristics of Clove Plants

Observations of the morphological characteristics of the clove plants were carried out in three locations: *Jombang*, *Nganjuk*, and *Trenggalek*, using the same variety, the Zanzibar variety. The observed morphological parameters included 16 characteristics, namely Crown Shape (CS); the stem characteristic that is the Stem Surface (SS), Number of Main Branches (NMB), and the Stem Color (SC); the characteristic of the leaf that is the Leaf Shape (LS), the Leaf Base Shape (LBS), the Leaf Apex Shape (LAS), Young Leaf Color (YLC), Mature Leaf Color (MLC), Leaf Surface Texture (LST), Leaf Length (LL), and Leaf Width (LW); the characteristic of the flower that is the Number of Flowers per inflorescence (NF), Young Flower Color (YFC), Mature Flower Color (MFC), and the Flower Shape (FS). Each of the morphological characteristics from the three locations is presented in Table 2.

Table 2. Morphological characteristics of clove plants in the three locations.

Morphological characteristics	**Location**		
	Nganjuk	**Jombang**	**Trenggalek**
Crown shape	Pyramid	Pyramid	Pyramid
Stem surface	Smooth	Scurfy	Scurfy
Number of Main Branches	Two Branches	Two Branches	Two Branches
Stem color	Brown	Gray	Brown
Leaf shape	Ovate	Ovate	Elliptic
Leaf base shape	Acute	Acute	Acute
Leaf apex shape	Rounded	Acute	Acute
Young leaf color	Deep Pink	Orange	Orange
Mature leaf color	Dark Green	Light Green	Dark Green
Leaf surface texture	Glabrous	Glabrous	Glabrous
Leaf length	10.36	12.42	13.2
Leaf width	3.9	4.02	4.4
Number of flowers/ Inflorescences	5	5	5
Young flower color	Light Green	Light Green	Light Green
Mature flower color	Red	Red	Red
Flower shape	Obovate	Obovate	Obovate

In this study, the observed clove plant varieties were identical, indicating similarities in morphological characteristics. The morphological characteristics showing similarities were eight, namely: (1) CS, (2) NMB, (3) LBS, (4) LST, (5) NF, (6) YFC, (7) MFC, (8) FS. The morphological characteristics that differed were eight, namely: (1) SS, (2) SC, (3) LS, (4) LAS, (5) YLC, (6) MLC, (7) LL, (8) LW.

The crown shape of the clove trees from the three locations is pyramidal, following the branching and twig pattern. Based on the research of Soenarsih, Wahyudiyono, and Mandea (2021) the shape of the clove crown of the Zanzibar variety is a pyramid. The crown is an integral part of the tree's structure; variations in its shape play a role in influencing the surrounding microclimate (Saroh & Krisdianto, 2020).

Stem is one of the main organs of a plant. Its most important function is to provide mechanical support. It means that the stem of a plant provides the necessary structure to support and keep the plant upright, allowing it to grow towards sunlight. In addition, it also acts as a link between various other plant organs. Both the leaves and the roots are directly connected to it. Air and nutrients from the roots to the leaves and sugars from photosynthesis to all parts of the plant are carried through the stem. It also supports leaves, flowers, and fruits (Putri, 2021). The stem surface of clove plants in *Nganjuk* is smooth, whereas in *Jombang* and *Trenggalek*, it is scurfy.

Variations in stem surface characteristics, such as the presence or absence of scurf, can be influenced by environmental factors, including humidity, temperature, and microbial interactions on the plant surface. Although the stems in Jombang and Trenggalek appear scurfy, this does not affect their overall growth habits, as all plants in all three locations show upright stem growth. According to Soenarsih et al. (2021) the upright stem orientation of clove plants is an adaptive trait that facilitates optimal light interception and efficient resource allocation, especially in open field conditions. The architecture of the upright stem is also important for supporting reproductive structures and maintaining the stability of the crown in various climatic conditions. Despite differences in surface texture, the consistency in the orientation of the stems indicates that the Zanzibar variety exhibits stable architectural properties in various environmental settings, strengthening its adaptability and structural robustness.

Leaves are the parts of plants that grow on stems or branches, usually green in color, and are most commonly found on plants. Leaves function in the process of photosynthesis, which is to process food substances (Mayoru et al., 2022). Observations of leaf components show changes in the shape of the leaf apex, the color of the young and mature leaves, as well as the length and width. The longest and widest leaves of clove plants are found in Trenggalek.

Differences in leaf morphology between locations can be attributed to microclimatic conditions, such as light intensity, temperature, and availability of soil nutrients, which affect leaf expansion and pigment development. The observed variations in leaf apex from rounded to tapered indicate potential adaptive responses to environmental stressors, such as water availability and sunlight exposure. Furthermore, the color changes between young and mature leaves, ranging from dark pink to orange in young leaves, and from light green to dark green in mature leaves, may reflect differing anthocyanin and chlorophyll concentrations, which are influenced by physiological age and environmental stimuli (Rahmawati et al., 2023). The larger leaf size observed in Trenggalek indicates favorable growth conditions that support greater leaf expansion, which has the potential to improve the efficiency of plant photosynthesis at the location.

In addition to vegetative properties, the morphology of the flowers was also examined and found to be relatively uniform across all study locations. The morphological consistency of the flowers suggests that the floral traits in Zanzibar varieties are more genetically stable than vegetative traits and are less affected by environmental variations. As reported by Reddappa, Sreekala, Reshma, and Deepa (2024) the uniformity of flowers in cloves is a desirable trait, as it supports the flowering period and maximizes yield potential.

The characteristics of the flower do not have a significant difference because they are still in the same variety. Flowers have several important functions in plant life, one of which is for reproduction. The flowers have both male and female reproductive organs that allow the fertilization process, which produces new seeds or fruits. Moreover, flowers also have a role in pollination, that is, the transfer of pollen from its source to the female reproductive organs, which is essential for seed formation. In addition to its reproductive function, flowers can also have a role in attracting insects, birds, or other animals that help with pollination, as well as give beauty and uniqueness to plants (Miftachurohman, 2017).

Based on the observation results, differences in morphological characteristics were found in the stem surface. The stem surface of clove plants in *Nganjuk* differs from that in other locations. This variation is due to the lower light intensity in *Nganjuk*. Daniel and Polanin (2013) stated that the main reason for the scurfy stem is that the trees have reduced foliation due to increased availability of sunlight, causing healthy bark/stem to continue to expand and peel off as the tree grows, with more cracks and uneven surfaces in older trees. Light intensity can also affect the color difference in the stems of clove plants in *Jombang*. Right there, the light intensity is higher than in the other locations. Based on this, different environmental conditions can cause differences in plant characteristics.

The leaf shape of clove plants in *Trenggalek* differs from other locations. Low temperatures with high humidity and low pH can affect the difference in the leaf shape in that place. This shows that different growing locations allow the plants to adapt to the prevailing environmental conditions. There is a difference in the shape of the leaf apex and the color of the young leaf of the clove plants in *Nganjuk* compared to other locations. This difference may be due to the low light intensity of Nganjuk's environment. Leaf size varies across locations. Variations in leaf size and shape are often affected by the weather. However, other factors such as the amount of light the plant receives or the nutrients available also play a role in differences in clove leaf morphology. Therefore, leaves can change size and shape depending on the surrounding environmental conditions (Li, Li, Zhang, & Li, 2015).

Observations show that the morphological characteristics of clove plants of the same variety, planted in different locations, exhibit morphological differences. This is very likely because each location has different environmental conditions, causing the plants to experience natural mutations. Natural mutation is a natural process that occurs in plants and contributes to genetic diversity. Environmental factors such as strong UV radiation, high temperatures, salt stress, water availability, light intensity, and other environmental factors can induce natural mutations (Ban & Jung, 2023).

The occurrence of such morphological divergence in genetically identical clove varieties underscores the phenotypic plasticity of the plant and its capacity to respond adaptively to environmental stressors. Over time, natural mutations affected by abiotic stress can become permanent in local populations, leading to the emergence of ecotypes or subpopulations that exhibit different morphological or physiological traits that adapt to a particular environment. This phenomenon is particularly relevant in perennials such as cloves, which live long and are constantly exposed to environmental variations. According to Putnins and Androulakis (2021), the accumulation of natural mutations under selective environmental pressure can be a source of new traits that may be useful for future breeding programs.

In this context, documenting morphological differences among clove populations across different ecological zones not only contributes to our understanding of intraspecific variation but also supports conservation and utilization strategies. Locally adapted traits that emerge through natural mutations can offer better resistance to climate change, pests, or diseases. Therefore, recognizing and preserving this phenotypic diversity is crucial for sustainable clove cultivation and long-term genetic improvement of the crop.

Results of Clove Plants PCA in the three locations.

In this study, PCA of the morphological characteristics of Zanzibar clove plants in *Nganjuk*, *Jombang*, and *Trenggalek* regions provides in-depth insights into the phenotypic diversity observed across these locations.

Figure 1 illustrates the relationship between growing locations (Nganjuk, Jombang, Trenggalek) and the morphological characteristics of clove plants.

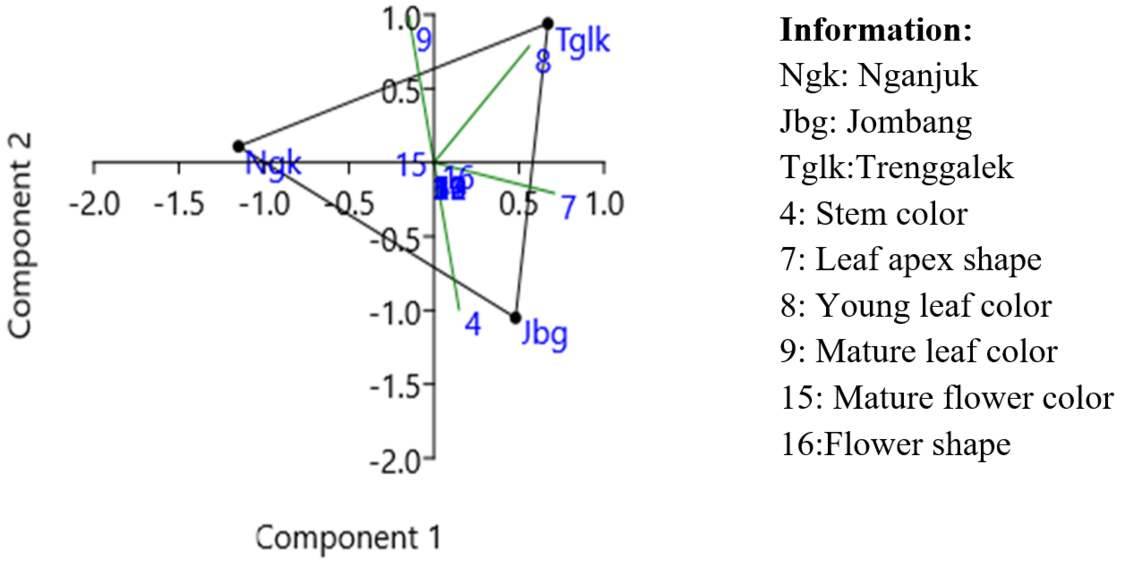

Figure 1. PCA results of scatter plots of clove plants in *Nganjuk*, *Jombang*, and *Trenggalek* locations.

Based on the results of the PCA scatter plot of morphological characters of clove plants, there are two principal components (PC), namely PC 1 and PC 2. PC 1 (x-axis) is located in the locations of Jombang and Trenggalek, which means that these two locations have a high similarity in the morphological characteristics of clove plants. PC 2 (y-axis) is located at Nganjuk, where, according to the research that has been carried out, it is a location with an altitude of more than 600 m above sea level, so the morphological characteristics in this location are only slightly similar to other locations. According to Nurhayati and Yusoff (2022), lowlands are more fertile for cloves than mountains. Cloves can still produce flowers from 0 to 900 meters above sea level (ASL), but flower production will decrease as altitude increases, although the plant itself may grow more luxuriously. Clove flowers bloom best at an altitude of 200–600 meters above sea level. If the soil has good drainage, clove plants thrive in coastal lowlands and hilly areas at an altitude of 600–1,100 meters above sea level (Enintha, 2022).

The results of the morphological characteristics analysis showed that there are significant differences in clove plants between the locations of *Nganjuk*, *Jombang*, and *Trenggalek*. It indicates that altitude not only influences growth but also the development of the clove plants. Based on the morphological data recorded at the three locations, there are significant differences in the characteristics of the Zanzibar variety clove plants.

Clove plants in *Jombang* showed a scurfy stem surface, grey stem color, and ovate leaf shape with a length of 12.42 cm and a width of 4.02 cm. On the other hand, clove plants in *Nganjuk* have a smooth stem, brown stem color, and darker young leaves, namely, deep pink. The clove plants in *Trenggalek* have a scurfy stem surface with the same stem color as in *Nganjuk*, that is brown, but have a different leaf shape, that is elliptic. From these differences, it can be concluded that environmental factors at each location play an important role in shaping the morphological characteristics of the clove plants of the Zanzibar variety.

The separation of *Nganjuk* from *Jombang* and *Trenggalek* based on the second principal component (PC2) demonstrated that altitude significantly influences the morphological diversity of clove plants. At higher altitudes, physiological responses to environmental stresses such as lower temperatures, increased humidity, and variations in light intensity can induce changes in leaf size, color, stem surface texture, and other adaptive traits. This observation aligns with the concept of ecotype differentiation, where populations of the same species display different phenotypic traits in response to local environmental pressures.

From an agronomic perspective, understanding the influence of altitude and environmental gradients on morphological traits is essential for site-specific clove cultivation strategies. For example, locations such as *Trenggalek* and *Jombang*, which show similar morphology and are within the optimal altitude range for flower production (200–600 m above sea level), can be prioritized for high-yield clove cultivation. In contrast, areas such as Nganjuk, although potentially less favorable for flower productivity due to its altitude above 600 m above sea level, may still offer advantages in terms of vegetative vigor and plant resilience, especially if superior clonal materials adapted to highland conditions are used.

These PCA results not only enhance our understanding of spatial morphological variation but also establish a foundation for further ecological and genetic studies. Future research integrating morphological, physiological, and molecular markers is necessary to determine whether the observed morphological differences are due to phenotypic plasticity or underlying genotypic divergence.

1. Flavonoid Content of Clove Leaves

The analysis of flavonoid content in clove leaves was conducted from three locations (*Nganjuk*, *Jombang*, and *Trenggalek*) using the UV-Vis spectrophotometry method.

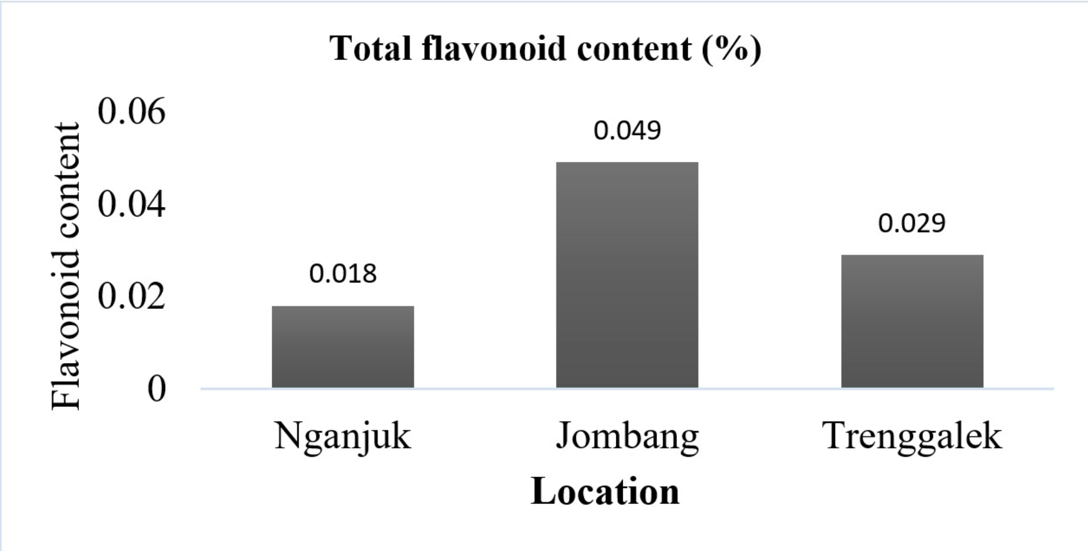

Figure 2. The average results chart of flavonoid content in Jombang, Nganjuk, and Trenggalek locations.

Based on the results of the analysis of flavonoid content from the three locations, the results were obtained, averaged, and presented in Figure 2. *Jombang* obtained an average result of 0.049%, *Nganjuk* obtained an average result of 0.018%, and *Trenggalek* obtained an average result of 0.029%. The highest flavonoid content was found in *Jombang*, at an altitude of 525 meters above sea level and with higher light intensity than other locations. Light intensity plays a crucial role in influencing plant survival, early growth phases, the development of the photosynthetic apparatus, and the production of secondary metabolites. This factor is key in maintaining plant vitality, supporting early growth stages, optimizing photosynthetic function, and stimulating the production of additional metabolites that contribute to adaptive responses and the synthesis of essential chemicals.

Flavonoid compounds are produced through the biosynthesis pathway, initiated by photosynthesis. During photosynthesis, plants convert solar energy into chemical energy in the form of sugars, such as glucose. This process occurs in chloroplasts and involves various enzymes as well as photosynthetic pigments, such as chlorophyll. Photosynthetic products, in the form of sugars or carbohydrate compounds, can enter the phenolic biosynthesis pathway. Here, early phenolic compounds, such as phenylalanine, are produced from the first amino acid. Phenylalanine is then converted to 4-Coumaroyl CoA through a series of enzymatic reactions involving enzymes such as phenylalanine ammonia-lyase (PAL) and coumarate: CoA ligase. 4-Coumaroyl CoA is combined with three molecules of malonyl CoA to form a chalcone, a key intermediate in the flavonoid biosynthesis pathway. This reaction occurs through the action of the enzyme chalcone synthase (CHS). Chalcone is an intermediate compound in the flavonoid biosynthesis pathway. Chalcone can undergo further modification into more complex flavonoids through a series of enzymatic reactions, including hydroxylation and condensation. Through complex biosynthesis pathways, chalcone can be converted into different types of flavonoids, such as flavonols, flavones, and anthocyanins. This process is influenced by various enzymes and the specific reaction pathways involved. The resulting flavonoid compounds can be stored in various parts of the plant, including leaves, flowers, and fruits (Zhang et al., 2021).

The Andosol soil type in the *Nganjuk* area is characterized by a loose soil structure, good drainage, and high mineral and organic matter content. The alluvial soil type in the *Jombang* area has a sandy clay texture that affects drainage and water retention. The Mediterranean mix soil type in the *Trenggalek* area contains high levels of clay (Afra, 2023).

Andosol soil type has fertile and optimal soil conditions for plant growth, which may reduce stress on plants; thus, plants do not need to produce high amounts of flavonoids as a defense mechanism. Alluvial soil type has nutrient-rich soil conditions; variations in soil texture can cause variations in water and nutrient availability. However, it may experience stress caused by changes in soil moisture and fluctuating nutrient availability, which can trigger the production of flavonoids in response to these stress conditions. The type of mixed Mediterranean soil has a high lime content, causing stress on plants because some nutrients may not be available optimally, thus producing flavonoids in response to the limitations of some nutrients (Montgomery & Biklé, 2021).

The observed differences in flavonoid content also support the hypothesis that the accumulation of secondary metabolites in clove leaves is influenced by a combination of genotype potential and local environmental conditions. Although all samples were from the same clove variety (Zanzibar), their chemical composition varied significantly across locations, reinforcing the concept of environmentally influenced biochemical plasticity. These findings are in line with the results reported by Bibi et al. (2022), which emphasize that flavonoid biosynthesis is highly responsive to environmental cues such as ultraviolet (UV) radiation, soil nutrient availability, and abiotic stress levels.

From a phytopharmaceutical and agronomic perspective, the higher flavonoid content in *Trenggalek* may enhance the functional properties of clove leaves harvested from the region, including antioxidant capacity, anti-inflammatory effects, and potential medicinal applications. These findings can serve as a basis for site-specific harvesting or selective breeding aimed at maximizing the yield of bioactive compounds. Furthermore, these data highlight the importance of an integrated agroecological management strategy that emphasizes not only crop yields but also the quality and biochemical richness of plant products.

In future studies, it will be useful to examine seasonal variations in flavonoid content, assess additional phytochemical markers, and explore correlations with soil parameters and microclimate data. Metabolomics approaches can also provide deeper insights into the biosynthetic pathways involved and their regulation under various environmental stresses. Such studies will be crucial for optimizing clove cultivation zones and post-harvest processing for the pharmaceutical, nutraceutical, and functional food industries.

4. CONCLUSION

Differences in growing locations and environmental conditions (*Nganjuk*, *Jombang*, and *Trenggalek*) influence the morphological characteristics of clove plants. Morphological characteristics that show differences between the three locations are stem surface, stem color, leaf shape, leaf apex shape, leaf color, and leaf size. The different growing locations of the plants also affect the flavonoid content of clove leaves. The highest flavonoid content was found in clove leaves grown in *Jombang*, at 0.049%.

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Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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