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RESEARCH PAPER

The Effects of Oxygen Availability in the Seed Container during Storage on Seed Germination in Tomato, Onion, Cabbage, and Marrow Seeds

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Abstract

This research was conducted to test the effect of oxygen content (low O_2, high O_2, air) during hermetic seed storage at 20±2°C over 8 and 12 months on seed germination and seedling root and shoot length in tomato, onion, cabbage, and marrow seeds. Samples with low oxygen storage had higher seed germination as well as longer root and shoot lengths than both control and high oxygen storage. When the storage period extended from 8 to 12 months, the germination percentages also reduced. However, these results varied among the species. The greatest advantage of low oxygen storage was obtained in tomatoes, which exhibited 15% and 9% higher germination compared to the control after 8 and 12 months of storage, respectively. The longest root and shoot lengths of 6.4 cm and 11.6 cm, respectively, were obtained from the low oxygen storage treatments. A similar positive effect of low oxygen storage was observed in onion and cabbage seeds but not in marrows. Results indicated that oxygen level in the packets during storage can be an effective component to maintain high seed germination and seedling growth potential (seed vigour). The difference in the effect on different species is a matter of further research.

1. Introduction

The storage environment greatly influences seed longevity. The principal environmental factors affecting seed deterioration and seed survival are temperature, seed moisture (relative humidity), and oxygen pressure (Ellis and Hong, 2007; Schwember and Bradford, 2011; Han et al., 2021). In orthodox seeds, the relationship between seed longevity, temperature, and moisture are determined over wide ranges in different species (Ellis and Roberts, 1980; De Vitis et al., 2020; Hay et al., 2022). Seeds of agronomic crops are in general produced in large amounts and stored in open storage (in cloth bags, paper bags etc.) where the seed moisture eventually equilibrates with ambient relative humidity and temperature, and oxygen is freely available at atmospheric concentration during storage. In vegetables however, particularly hybrid cultivars, seeds are very valuable and are mostly kept in hermetic packets in rather small amounts compared to cereals or agronomic crop seeds. Here, the gaseous environment in the packets can be regulated during the storage. The role of oxygen in seed storage has not been investigated as much detail as seed moisture or temperature, since there was an assumption that its effect on longevity in air dry storage was modest. The importance of oxygen on seed storage was first emphasized by Roberts and Abdalla (1968) in rice. More recently however, there are an increasing number of studies in which the deleterious effect of oxygen on longevity has been clearly demonstrated (Barzali et al., 2005; Gonzales-Benito et al., 2011; Schwember and Bradford, 2011; Groot et al., 2012; Groot et al., 2015; Buijs et al., 2020; Pirredda et al., 2020; Prasad et al., 2022; Tahir et al., 2023). The

damaging effect of oxygen on seed germination during storage has been associated with damage to macromolecules (DNA, RNA and proteins) (Sano et al., 2016), ROS enzymes and lipid peroxidation (Tahir et al., 2023), and accumulation of point mutations (Kranner et al., 2011).

Seed deterioration and ageing during storage have always been of major concerns in plant production. Vegetable seeds such as tomatoes, cabbages, and marrows are produced through transplants, and very small differences in seed germination during storage can result in great differences in their performance in the greenhouse or field. Seed vigour describes overall performance of the seed lots in a non-optimum sowing environment (ISTA, 2022). Seed vigour denominates the differences in performance of those seed lots that have similar seed germination percentages in stress environments. Therefore, it can be an important measurement criterion to obtain strong transplants for plant production. In this study, we investigated the effect of oxygen content in the packets on seed germination and vigour in tomato, cabbage, onion, and marrow seeds.

2. Material and Methods

The seeds of tomato (*Solanum lycopersicum* cv. Falcon), onion (*Allium cepa* cv. Elit), cabbage (*Brassica oleracea* var capitata cv. Yalova), and marrow (*Cucurbita pepo* L. cv. Sakız) were obtained from commercial seed companies. Total seed germination percentages were recorded as 91, 93, 84, and 100% in tomato, onion, cabbage and marrow seeds, respectively. The seed moisture content of the seeds tested was determined as 7.0, 7.7, 6.2, and 6.6%. Initial laboratory germination tests of seeds were conducted with three replicates of 50 seeds in each species. The seeds were placed between wet filter paper (10 ml distilled water, 20 × 20 cm, Filtrak, Germany). The papers were then placed in plastic bags. Germination tests were carried out in the dark at 20°C for onions and cabbages and 25°C for tomatoes and marrows. Standard germination (normal and abnormal seedlings) was evaluated after 14 days for tomatoes, after 12 days for cabbages and onions, and seven days for marrow seeds. Seeds that produced a 2 mm radicle emergence were counted every day at the same time and considered as total germination (TG). At the final count, normal seedling percentages (NG, seedlings with developed root and shoots) were also determined (ISTA, 2022).

Six samples were prepared for each species, each containing 150 seeds. A total of 900 seeds for each species were weighed and equilibrated to 75% relative humidity in plastic cups (360 mm long × 180 mm wide × 120 mm deep) by placing them on top of wire mesh over a saturated NaCl solution (41 gram per 100 ml) for three days at room temperature in the dark. At the end of the equilibration, the seed moisture content at which seeds were stored was calculated by weighing (Basak et al., 2006). The seed moisture contents of the seeds at storage were 10.6, 12.4, 9.6, and 8.2% for tomato, onion, cabbage and marrow, respectively. A hundred and fifty seeds per sample were placed in a glass jar (22 ml) and low O_2 (Nitrogen) nitrogen (Kasweld EN ISO 2503) or high oxygen gas (100% High O_2) was added by using oxygen (UN1072, Kasweld oxygen EN ISO 2503) tubes. The caps of the jars were closed tightly and covered well with cling film. The control seeds were stored in air (21% O_2). The samples were placed at 20±2°C in the dark plastic bags. Seed quality tests were conducted in three samples (control, low O_2, high O_2) of each species. Germination tests after each storage duration or period were conducted as described above, and total and normal seed germination percentages were determined. At the end of the germination tests, 15 seedlings (five seedlings × three replicates) were selected randomly, and root and shoot lengths measured as cm in each treatment sample, species and storage period.

Means of total and normal germination percentages and shoot and root length in the seed samples in each species were compared at the 5% level by Duncan's multiple range tests by using the SPSS package program (IBM version 25). Angular transformation for percentages was carried out before analyses.

3. Results and Discussion

Total germination percentages (radicle emergence, 2 mm) after the fourth-day or the final count were not significantly different in either the treatments or in any species (Table 1) after eight and 12 months of storage. A single significance in the fourth-day radicle emergence percentage was observed in high O_2 storage in onion seeds after eight months compared to the other two treatments. In this sample, radicle emergence percentages declined to 55%, while control and low O_2 were 69 and 68%, respectively (Table 1).

Low oxygen storage influenced normal seed germination percentages in tomatoes and cabbages in both storage periods. In onions, the effect was clearer at eight months of storage but was not significant after 12 months. Neither the control nor any of the oxygen content levels exhibited significant differences in marrow seeds during the storage period. When tomato seeds were stored at low oxygen level, seed germination was 15 and 18% higher after eight months than that of the control and high oxygen stored seeds (P<0.05). Corresponding differences in 12 months of storage were 9 and 10%, respectively (Table 1). Even though low oxygen-stored cabbage seeds had the highest seed germination values of 71 and 75%

Table 1. Changes in radicle emergence (2 mm long) of 4th day and final counts of tomato, onion, cabbage, and marrow seeds stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 8 and 12 months.

Species	4th day radicle emergence percentage					
	8 month			12 month		
	Control	High O_2	Low O_2	Control	High O_2	Low O_2
Tomato	80±8.0 a*	80±2.0 a	87±5.8 a	76±3.5 a	76±5.3 a	82±6.9 a
Onion	69±1.2 a	55±8.1 b	68±7.2 a	54±2.0 a	40±13.1 a	47±3.1 a
Cabbage	78±8.0 a	78±2.0 a	79±8.3 a	74±3.5 a	75±3.1 a	80±7.2 a
Marrow	95±2.3 a	98±2.0 a	95±2.3 a	99±1.2 a	99±1.2 a	99±1.2 a
Species	Final count radicle emergence percentage					
	8 month			12 month		
	Control	High O_2	Low O_2	Control	High O_2	Low O_2
Tomato	92±6.9 a	92±3.5 a	97±2.3 a	90±5.3 a	91±4.2 a	94±5.3 a
Onion	91±4.2 a	91±1.2 a	93±4.6 a	87±1.2 a	79±7.0 a	79±2.3 a
Cabbage	81±9.0 a	81±5.0 a	84±8.0 a	79±3.1 a	80±3.5 a	85±9.9 a
Marrow	95±2.3 a	98±2.0 a	95±2.3 a	99±1.2 a	99±1.2 a	99±1.2 a

* Mean values were given with SEM and the means with different letters in the same line and species in the same storage period were significantly different (*P<0.05*).

Table 2. Changes in normal germination percentages, 4th day root length and shoot length of tomato, onion, cabbage, and marrow seeds stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 8 months.

Treatments	Germination (%)			
	Tomato	Onion	Cabbage	Marrow
Control	77±4.2 b*	73±2.3 b	66±7.2 a	85±4.6 a
High O_2	74±5.3 b	74±2.0 b	65±5.0 a	86±3.5 a
Low O_2	92±3.5 a	84±3.5 a	75±8.1 a	87±4.2 a
Treatments	Root length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	3.2±1.1 b	3.9±0.8 b	6.1±1.7 a	5.4±0.8 b
High O_2	3.5±1.5 b	3.7±0.9 b	3.7±1.0 c	6.0±0.6 a
Low O_2	6.4±1.6 a	5.2±0.8 a	5.0±1.4 b	5.2±0.7 b
Treatments	Shoot length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	8.5±1.6 b	6.2±0.7 b	6.2±0.6 a	3.6±0.6 a
High O_2	7.6±1.5 b	5.1±0.9 c	4.5±0.8 b	3.9±0.6 a
Low O_2	11.6±1.2 a	7.7±1.1 a	6.8±1.1 a	3.5±0.6 b

* Mean values were given with SEM and the means with different letters in the same column and species in the same criterion were significantly different (*P<0.05*).

after eight and 12 months of storage, the differences between the control and high oxygen content level stored seeds were not statistically significant. Onion seeds that were stored at low oxygen level had the highest value of 84% among the treatments after eight months, but the difference was in favour of control seeds, at 71% after 12 months of storage. Thus, onion seed germination was more changeable compared to tomato and cabbage seed (Table 1 and 2) as the seed storage period was extended. The normal seed germination of marrow did not vary significantly when stored under conditions of air, low oxygen or high oxygen. Seed germination varied between 85 and 87% after eight months and 77 and 84% after 12 months of storage. There was no statistically significant difference among the treatments after either eight or 12 months (Table 2 and 3).

Earlier studies on the effect of oxygen on longevity have indicated that oxygen plays an effective role in seed germination in barley, peas and broad beans (Roberts and Abdalla, 1968), lettuce (Ibrahim et al., 1983), rye (Barzali et al., 2005), sesame (Ellis and Hong, 2007), onion (Schwember and Bradford, 2011), brassica species (Gonzales-Benito et al., 2011), celery (Groot et al., 2015) and, rice (Tahir et al., 2023) seeds. It appears that the effect of low oxygen was seen to be positive in various type of plant seeds. These findings are in agreement with our results (Table 2 and 3). However, the species react differently. Our results indicated that marrow seeds were not affected by the presence of oxygen during storage, while tomatoes had the most positive response to reduced oxygen storage (Table 3). Obviously, there are various factors that may be responsible for that. One may be the chemical composition of the seed, as marrow is very oily and the seeds are larger as compared to the other seeds.

Secondly, seed coat structure can be a factor as marrow seed coat is harder in relation to water and gaseous exchange between the seed and its environment. Some earlier papers indicated such effects in controlled gas atmospheric storage conditions (Gonzales-Benito et al., 2011; Han et al., 2021). Ellis and Hong (2007) indicated that the effect of oxygen was more prominent at lower seed moisture contents than at higher contents. In this study, we equilibrated the seeds with saturated NaCl solutions (75% RH), which gave about 10.6, 12.4, 9.6, and 8.2% of seed moisture in tomato, onion, cabbage, and marrow. These moisture

Table 3. Changes in normal germination percentages, 4th day root length, and shoot length of tomato, onion, cabbage, and marrow seeds were stored at 20±2°C in the glass jars with low and high O_2 and air (control) over 12 months.

Treatments	Germination (%)			
	Tomato	Onion	Cabbage	Marrow
Control	76±2.0 ab*	71±3.1 a	66±2.0 a	81±3.1 a
High O_2	75±7.0 b	60±3.5 a	62±8.7 a	84±6.0 a
Low O_2	85±5.0 a	61±11.4 a	71±11.0 a	77±3.1 a
Treatments	Root length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	3.2±0.8 b	3.9±0.9 b	5.0±1.0 a	4.7±1.1 a
High O_2	3.5±1.1 b	3.5±0.7 b	3.6±0.6 b	4.6±0.9 a
Low O_2	5.0±2.0 a	4.6±0.7 a	4.6±1.2 a	4.4±0.5 a
Treatments	Shoot length (cm seedling^{-1})			
	Tomato	Onion	Cabbage	Marrow
Control	7.9±1.0 b	5.8±0.7 b	5.2±0.5 a	3.0±0.5 a
High O_2	6.7±1.3 c	5.0±0.7 c	3.7±0.4 b	3.0±0.5 a
Low O_2	8.8±1.0 a	6.9±0.8 a	5.5±1.1 a	3.2±0.4 a

* Mean values were given with SEM and the means with different letters in the same column and species in the same criterion were significantly different (*P<0.05*).

contents are relatively higher than what is used in commercial storage.

Schwember and Bradford (2011) observed the negative influence of oxygen presence at higher seed moistures in lettuce and onion seeds during artificial ageing. Tahir et al. (2023) also mentioned that at higher moisture levels (14%) the availability of oxygen in storage is more harmful to rice seed lifespan than is low seed moisture level (12%). It appears to be that oxygen may have an effect at any seed moisture, but the relative impact may be different (Groot et al., 2015). High seed moisture and the natural proportion of oxygen available during storage may accelerate the ageing process of the seeds and negatively affect seed germination as explained by De Vitis et al. (2020). The deteriorative effect of oxygen on seed germination was determined to be related to damage to macromolecules (DNA, RNA and proteins) (Sano et al., 2016), ROS (Tahir et al., 2023), and the accumulation of point mutations (Kranner et al., 2011).

The current study indicated that the root and shoot lengths were higher in seeds stored with low oxygen compared to both controls and those with high oxygen in tomatoes and onions after 8 and 12 months of storage (Table 2 and 3). The same as to normal germination percentages lower oxygen levels affected root and shoot lengths more in tomato and onion seeds than in cabbage and marrow seeds (Table 2 and 3) after both storage periods. In these two species, low O_2 storage increased both root and shoot lengths significantly (P<0.05) compared to control and high O_2 storage. The difference varied, but in tomato, it went up to 3.2 and 3.1 cm in low O_2 compared to that of the control after eight months of storage (Table 2). The difference in onion seeds was lower. In cabbage and marrow seeds, decreased oxygen composition had no effect on either root or shoot lengths. The differences in seedling lengths indicated that lowering the oxygen content increased seed vigour as reflected in seedling size. Vegetables are produced through transplants, and longer root and seedling size result in better stand establishment and larger seedling size (Demir et al., 2008). Therefore, the present results, which show a more positive effect on plant size in low oxygen storage may be valuable in preserving seed vigour, particularly for vegetable seeds in which large industrial transplant production is important (Zulfikar, 2021).

4. Conclusion

This study indicated that lower oxygen content during storage affected seed germination, and vigour (normal germination, seedling root and shoot length) in tomato, onion, and cabbage seeds. The effect was not pronounced in marrow seeds. It appeared to be that the effect of oxygen was somehow species dependent. Meanwhile, further research on the topic combining various seed moisture and temperature environments need to be explored in the future experiments.

References

Barzali, M., Lohwasser, U., Niedzielski, M., & Börner, A. (2005). Effects of different temperatures and atmospheres on seed and seedling traits in a long-term storage experiment on rye (*Secale cereale* L.). *Seed Science and Technology*, 33:713–721.

Basak, O., Demir, I., Mavi, K., & Matthews, S. (2006). Controlled deterioration test for predicting seedling emergence and longevity of pepper (*Capsicum annuum* L.) seed lots. *Seed Science and Technology*, 34:723-734.

Buijs, G., Willems, L.A.J., Kodde, J., Groot, S.P.C., & Bentsink, L. (2020). Evaluating eppo method for seed longevity analyses in Arabidopsis. *Plant Science*, 301:110644.

Demir, I., Ermis, S., Mavi, K., & Matthews, S. (2008). Mean germination time of pepper seed lots (*Capsicum annuum)* predicts size and uniformity of seedlings in germination tests and transplant modules. *Seed Science and Technology*, 1:21-30.

De Vitis, M., Hay, F.R., Dickie, J.B., Trivedi, C., Choi, J., & Fiegener, R. (2020). Seed storage: maintaining

seed viability and vigour for restoration use. *Restoration Ecology*, 28: S249–S255.

Ellis, R.H., & Roberts, E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany*, 45:13–30.

Ellis, R.H., & Hong, T.D. (2007). Seed longevity – moisture content relationships in hermetic and open storage. *Seed Science and Technology*, 35:423–431.

Gonzales-Benito, M.E., Perez-Garcia, F., Tejeda, G., & Gomez-Campo, C. (2011). Effect of the gaseous environment and water content on seed viability of four *Brassicaceae* species after 36 years storage. *Seed Science and Technology*, 13:443-451.

Groot, S.P.C., Surki, A.A., De Vos, R.C.H., & Kodde, J. (2012). Seed storage at elevated partial pressure of oxygen, a fast method for analysing seed ageing under dry conditions. *Annals of Botany*, 110:1149–1159.

Groot, S.P.C., De Groot, L., Kodde, J., & Van Treuren, R. (2015). Prolonging the longevity of ex situ conserved seeds by storage under anoxia. *Plant Genetic Resources*, 13:18-26.

Han, B., Fernandez, V., Pritchard, H.W., & Colville, L. (2021). Gaseous environment modulates volatile emission and viability loss during seed artificial ageing. *Planta*, 253:106.

Hay, F.R., Rezaei, S., & Buitink, J. (2022). Seed moisture isotherms, sorption models, and longevity. *Frontiers in Plant Science*, 13.

Ibrahim, A.E., Roberts, E.H., & Murdoch, A.J. (1983). Viability of lettuce seeds. *Journal of Experimental Botany*, 34:631-640.

ISTA. (2022). International Rules for Seed Testing. International Seed Testing Association, Welliselen, Switzerland.

Kranner, I., Chen, H., Pritchard, H.W., Pearce, S.R., & Birtic, S. (2011). Internucleosomal DNA fragmentation and loss of RNA integrity during seed ageing. *Plant Growth Regulation*, 63:63–72.

Pirredda, M., Gonzales-Benito, M. E., Martin, C., & Mira, S. (2020). Genetic and epigenetic stability in rye seeds under different storage conditions: ageing and oxygen effect. *Plants*, 9:393.

Prasad, C.T.M, Kodde, J., Angenent, G.C., De Vos, R.C.H., Diez-Simon, C., Mumm, R., Hay, F.R., Siricharoen, S., Yadava, D.K., & Groot, S.P.C. (2022). Experimental rice seed aging under elevated oxygen pressure: Methodology and mechanism. *Frontiers in Plant Science*, 13:1050411.

Roberts, E.H., & Abdalla, F.H. (1968). The influence of temperature, moisture and oxygen on period of seed viability in barley, broad beans, and peas. *Annals of Botany*, 32:97–117.

Sano, N., Rajjou, L., North, H.M., Debeaujon, I., Marion-Poll, A., & Seo, M. (2016). Staying alive: Molecular aspects of seed longevity. *Plant and Cell Physiology*, 57:660–674.

Schwember, A.R., & Bradford, K.J. (2011). Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. *Seed Science Research*, 21:175–185.

Tahir, A., Afzal, I., Khalid, E., Razzaq, M., & Arif, M.A.R. (2023). Rice seed longevity in the context of seed moisture contents and hypoxic conditions in the storage environment. *Seed Science Research*, 33:39-49.

Zulfikar, F. (2021). Effect of seed priming on horticultural crops. *Scientia Horticulturae*, 286:11097.

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RESEARCH PAPER

Investigating Phenotypic Diversity in a Germplasm Collection of Scarlet Eggplant under Mediterranean Conditions

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Abstract

This study aimed to determine phenotypic diversity in the germplasm collection of *Solanum aethiopicum*, also known as scarlet eggplant, under Mediterranean conditions. Two different experiments were established in which morphological and valuable agronomic traits were employed to measure diversity among 57 and 55 accessions, respectively. The experiments were carried out in a greenhouse and open field, and descriptors designated by the European Cooperative Program for Plant Genetic Resources (ECPGR) and the International Board for Plant Genetic Resources (IBPGR) were used to measure the plants and fruits. The results from descriptive statistics on quantitative traits data of plants and fruits show a great variation among accessions of *Solanum aethiopicum*. Multiple correlation analysis in the two distinct experiments shows that the highly correlated variables/descriptors represented fruit quantitative traits. Finally, results from principal component analysis (PCA) confirm that the overall differences observed in the germplasm collection of *Solanum aethiopicum* were mainly due to fruit quantitative traits, which are decisive for phenotypic characterization of this eggplant.

1. Introduction

Scarlet eggplant is often described as African eggplant. However, African eggplant is recognized as a large group in which African native eggplants such as gboma eggplant (*Solanum macrocarpon* L.) as well as scarlet eggplant (*Solanum aethiopicum* L.) are comprised as well as their wild ancestors (*Solanum dasyphyllum* and *Solanum anguivi*), respectively. In African cuisine, African eggplants are appreciated mainly for their edible fruits and sometimes for their leaves (Haliński et al., 2017; Mibei et al., 2018). Furthermore, they are commercially significant, and they are particularly popular among Sub-Saharan African smallholder farmers since they are effortless to grow with minimal inputs and are altogether matched to the local environment (Weller et al., 2015; Sseremba, 2019).

Scarlet eggplant (*Solanum aethiopicum* L.), broadly acknowledged as the other cultivated eggplant, is a near relative of Brinjal eggplant (*Solanum melongena*) and one of its direct wild ancestors is *Solanum anguivi*. Furthermore, a wide number of wild relatives of Brinjal eggplant are found on the African continent (Knapp et al., 2013; Knapp et al., 2019). Scarlet eggplant is a diploid with 24 chromosomes (2n = 24) and also part of the genus *Solanum* (Shimira et al., 2021). The genus *Solanum* is vast and prosperous in species. About 1400 species are approximately estimated to exist in this rich genus. Thirteen principal clades exist within the genus such as the spiny solanums, the Leptostemonum clade and the Potato clade (which comprises of potato and tomato) just to name a few.

The scarlet eggplant (*S. aethiopicum* L.) is part of the “Anguivi clade” (Knapp et al., 2013; Knapp et al., 2019). Scarlet eggplant is described as an

herbaceous shrub with glabrous or hairy leaves. It is distinguished by its hermaphroditic flowers that grow in clusters or separately and these flowers are self- or cross-pollinated (Kamga et al., 2015). Scarlet and gboma eggplants are essentially cultivated and grown on the African continent, while the common eggplant is omnipresent globally; including Africa, Asia, subtropics (Central America, India) and in tempered regions like Mediterranean and southern USA (Gramazio et al., 2016; Zhuang et al., 2012). Apart from West and Central Africa, *S. aethiopicum* is also present in Caribbean countries, Brazil as well as South Italy. Africa is homeland to a great variety of wild relatives of the common eggplant (*Solanum melongena* L.) (Knapp et al., 2013; Gramazio et al., 2016).

Like common eggplant (brinjal), scarlet eggplant (*Solanum aethiopicum* L.*)* is consumed raw, boiled and/or fried, and also as an ingredient in stew and soup (Eletta et al., 2017). Their bitter taste is praised in their leaves and fruits. To a great extent, this may be due to the occurrence of alkaloids (mainly glycolalkaloids and phenolic compounds) establishing their edibility. In sub-Saharan Africa, leaves are consumed as vegetables, particularly leaves of the *S. aethiopicum* Kumba and Shum groups, ones of the four groups that make up *Solanum aethiopicum*. Consumers' preference for fresh fruit relies on a few quality traits such as fruit acidity and taste, fruit color, and phenolic contents in fruit and fruit epidermis (Adeniji et al., 2012).

There is mounting evidence that eating its leaves and fruits decreases the occurrence of chronic diseases such as diabetes and atherosclerosis (Mibei et al., 2018). The common eggplant (*Solanum melongena*) fruits have been used for the treatment of different diseases such as asthma, arthritis, bronchitis, and diabetes, as well as its nutritional features is valuable to the human diet (Foo et al., 2018). In the same manner, the African eggplant *Solanum aethiopicum* and *Solanum macrocarpon* have been used in traditional medicine in the cure of allergic rhinitis, asthma, constipation, dyspepsia, gastro-esophageal reflux disease, nasal catarrh, rheumatic disease, skin infections, and swollen joint pains. They are also used in weight reduction (Eletta et al., 2017). Furthermore, Kamga et al. (2015) have stated that the fruit from the three edible groups (Gilo, Kumba, and Shum) may accommodate up to 2% potassium, 10% protein, 80% water, high levels of antioxidant compounds, and carotenoids. It has been also reported by Kamga et al. (2013) that the Oforiwa variety of *Solanum aethiopicum* (originated from Ghana) contains a higher amount of carotenoids compared to other *S. aethiopicum* eggplant varieties originated from Ghana.

A small number of studies on the scarlet eggplant (*Solanum aethiopicum L.*) have been conducted in Africa and in Rwanda in particular. According to reports, eggplants are Rwanda's fifth most produced vegetable after cabbages, tomatoes, fresh beans, and squashes. Additionally, they are grown under the mixed agricultural practice that characterizes vegetable cultivation throughout the country, and most of the time on limited plots (Larochelle and Alwang, 2014; Van Dijk and Elings, 2014; Prasad et al., 2016). Eggplants are cultivated from commercial seeds accessible in the country, and their aspect is generally mediocre (Van Dijk and Elings, 2014). The hybrid seed market remains modest, and the seed types offered are insufficient. A few private companies sell open-pollinated varieties at reasonable prices in various franchise shops across the country (Dijkxhoorn et al., 2016; Uwamahoro et al., 2020).

The informal seed sector is dominant in Rwanda, with farmers primarily using saved seeds. Adoption of enhanced crop varieties is relatively moderate, with only a few breeding lines and improved varieties of African eggplant documented. Maize is an exception, with high adoption of hybrids. The Rwanda Agriculture Board primarily produces breeds and pre-basic seeds of crops such as maize, beans, rice, and wheat. National breeding programs focus on economically important crops such as sweet potatoes, cassava, and common beans, with a focus on developing pest and virus-resistant varieties through marker-assisted selection. As well as potato clones crops resulting from crosses between local and foreign varieties have been released in the country (Gapusi et al., 2013; Larochelle and Alwang, 2014; Context Network, 2016; Mujuju, 2018; Dusengemungu et al., 2019; Shimira et al., 2020). No African eggplant varieties or seed products have been released by national agricultural research institutions since 1990, with all available varieties being open-pollinated (Schreinemachers et al., 2017).

Globally, substantial investigations and breeding programs have been conducted on brinjal (*Solanum melongena* L.) and, to a lesser extent, on its related species such as *Solanum americanum*, *Solanum incanum*, and *Solanum torvum* (Isshiki et al., 1998; Caguiat and Hautea, 2014). This condition dragged scarlet eggplant (*Solanum aethiopicum* L.) and relatives (close and/or wild) into the category of "orphan crops" or underappreciated crops, along with a great number of other African indigenous vegetables (Kamga et al., 2015; Weller et al., 2015; Song et al., 2019). Scarlet eggplant, along with *Solanum incanum*, is acknowledged as a desirable provenance of variations in *Solanum melongena* breeding schemes, and it has also been revealed as cross-compatible with *Solanum melongena* (Gramazio et al., 2016). In brief, due to environmental changes in recent years, there is in breeding programs an enthusiasm for crop wild relatives (CWR) of agronomically important crops. These taxa have wide-raging relationships and identities (Knapp et al., 2013).

Within the *Solanum aethiopicum* species, four cultivar groups were identified based on morphological characteristics: Gilo, Aculeatum,

Kumba, and Shum. Thus, the Gilo group is by far the most significant of these groups, and it is appreciated for its tasty oval to spherical fruits (Osei et al., 2010; Adeniji et al., 2012; Kamga et al., 2015; Gramazio et al., 2016; Haliński et al., 2017). The only group that is not edible is the Acelatum group (Kamga et al. 2015). As also described by Lester and Daunay (2003), different shapes and sizes of fruits and leaves subsist within the four cultivars of *Solanum aethiopicum*.

Smallholder farmers' ability to grow and realize benefits from scarlet eggplant and other African indigenous vegetables (AIVs) is limited by a scarcity of high-quality seeds. Additionally, attempts to assist AIVs are impeded by an absence of breeding, genetics, and market demand research (Kansiime et al., 2018). A few number of investigations on the scarlet eggplant (*Solanum aethiopicum* L.) have been conducted in Rwanda. As a case in point, Adeniji et al. (2012) employed one accession of the *Solanum aethiopicum* Gilo group from Rwanda in a phenotypic diversity research with 43 other scarlet eggplant accessions from Africa, Europe, Asia, and South America (inclusive of all four groups: Gilo, Shum, Kumba, and Aculeatum). Similarly, in a morphological characterization research of gboma eggplant, scarlet eggplant, and wild related (*Solanum anguivi*) accessions from five additional African nations, Kamga et al. (2015) employed three Rwandan scarlet eggplant accessions.

During the domestication of *Solanum aethiopicum* from its ancestral parent, *Solanum anguivi*, distinctive gains or morphological diversity have been recorded. Changes in leaf sizes and shapes from broad, deeply lobed, hairy, prickly, shrubs, perennial plant and spherical fruit (1 cm diam.) to smaller, less lobed leaves, glabrous, non-prickly, herbs, annual plants and different shaped and sized fruit. Obtained cultigens resulting from domestication of *Solanum aethiopicum* displays different morphological features with their ancestor and within themselves (Lester and Daunay, 2003). This morphological divergence was also confirmed by Adeniji et al. (2012) when they carried out diversity examination among the *Solanum aethiopicum* groups. Higher divergence was reported in different accessions within the Gilo, Aculeatum, and Kumba groups than between groups themselves. However, a little difference (minimum diversity) has been perceived when analyzing molecular markers, essentially isozymes and DNA between cultigens (Lester and Daunay, 2003).

In brief, the intent of this study is to investigate the phenotypic diversity of scarlet eggplant under Mediterranean conditions. This will be achieved through the determination of the morphological diversity of several eggplant accessions originated from Rwanda using several descriptors of Solanaceae and eggplants. Additionally, the study aims to select and recommend ideal and high-potential accessions for future breeding programs. By understanding the diversity within this eggplant variety, we hope to contribute to the improvement of eggplant breeding and cultivation in Mediterranean regions.

2. Material and Methods

2.1. Plant materials

For the purpose of this research study, different experimental fields (open-field, and greenhouses) were used to establish an eggplant germplasm field in Türkiye (Table 1) from seeds originally collected from two distinct districts (Gakenke and Musanze) of Rwanda (Shimira et al., 2021). Experimental fields were set up depending on the targeted specific morphologic experiments and the growing seasons (2020-2021, and 2022).

At each experimental field site, seed germination and propagation were carried out by sowing eggplant seeds from all 60 different accessions in plant growing trays (4 × 6 cells each) containing a mixture of peat and perlite (3:1, w/w). Four different eggplant accessions were sown on each plant's growing tray. The temperature in the greenhouse was kept at 25°C during the germination period, and the plant growing trays were frequently irrigated. Unfortunately, due to the poor quality of some seeds, all accessions did not develop as expected. Depending on the experimental sites and growing seasons, between 55 and 57 accessions germinated successfully.

2.2. Experimental design and plantlets transfer to the fields

In the seventh week after seed sowing and germination, well-grown eggplant plantlets were transferred from plant growing trays into a well-prepared and designated experimental field bed with natural soil. Plants were cultivated in a completely randomized block design, with three replications of each accession arranged within eight

Table 1. Location of experimental fields in Antalya (Türkiye).

N°	Institution	GPS coordinates	Types of cultivation	Growing season
1	Batı Akdeniz Agricultural Research Institute (BATEM) - Department of Vegetable Crops and Ornamentals.	36°55'45.7"N 30°58'47.3"E	Greenhouse	2020-2021
2	Fidesan Fide Ltd.	36°54'38.0"N 30°58'25.8"E	Open field	2022

rows. The spacing between rows was approximately 1.4 m, and the spacing within rows was approximately 0.75 m. Water soluble fertilizers such as potassium nitrate (13-0-46), Mono-ammonium phosphate (12-61-0), and Urea were applied to the plants once a week. Drip irrigation was adopted, and irrigation is always performed on a regular basis to maintain soil moisture. Weeds were also thoroughly removed once per week. Other agronomic management practices were carried out, such as systematic pruning for each plant and application of chemicals in order to manage pests (Spider mite, *Tuta absoluta*, whitefly) and diseases (powdery mildew and botrytis).

Experiments of morphological characterization were both carried out in Antalya (Türkiye). The greenhouse experiment of agro-morphological characterization was performed at the BATEM Institute in spring-autumn from June 2020 to January 2021. The experimental field was 0.32 da and plant pruning was also carried out. The open field experiment of agro-morphological characterization was carried out in a field belonging to a private company (Fidesan Fide Ltd.) in spring-autumn from March 2022 to July 2022 (Figure 1). The experimental field in this case was also 0.32 da, however, there was no pruning carried out during this experiment.

2.3. Morphological variation analysis

The morphological and agronomic valuable traits assessed were mainly based on two distinct descriptors with some modifications. Those descriptors were both advanced by the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the International Board for Plant Genetic Resources (IBPGR) for *Solanaceae* and eggplant, respectively (Boyaci et al., 2020). Measurements on plant agronomic, qualitative, and quantitative traits (leaf prickliness, leaf hairiness, corolla color, plant height, etc.) were gathered according to replications. Similarly, fruit qualitative and quantitative traits (fruit color, length, weight, width, etc.) were measured using 3 ripened fruits (commercial stage) per replication of each accession. The used morphological and agronomically important traits are listed in Table 2, Table 3, and Table 4. Additionally, Figure 2, Figure 3, Figure 4, Figure 5 depict the work flow of agro-morphological characterization.

2.4. Multivariate analysis of morphological relationship

Relationship analysis was carried out on morphological traits data from two distinct growing seasons (2020 and 2022) by employing JMP software (version 15.2.1, SAS Inc., Cary, NC, USA). Furthermore, inter-trait correlations, principal component (PCA) and cluster analyses were determined by using the same software.

3. Results and Discussion

A perceptible level of phenotypic variations has been recorded from *Solanum aethiopicum* gr. Gilo germplasm in three distinct experiments with reference to measured morphological and valuable agronomic traits.

3.1. The greenhouse experiment (I)

A summary of the descriptive statistics (means, standard deviations, and maximum and minimum values) for the greenhouse experiment conducted in 2020 is shown in Table 5.

Descriptive analyses made from the data of quantitative traits of plants and fruits displayed a wide range of variations among all 57 accessions of *Solanum aethiopicum* gr. Gilo. For instance, the minimum leaf blade width was 14.7 cm (for accession MZE37) and the maximum was 31.7 cm (for accession MZE31); leaf blade length ranged from 24.0 cm (for accession MZE34) to 40.5 cm (for accession GKE14); and total plant height was scaled from 70.0 cm (for MZE27) to 240.0 cm (for GKE21).

Besides, we also noted extent changes from fruits' qualitative traits on studied accessions. As an illustration, fruit weight ranged from 10 g (for

Figure 1. Open field at Fidesan Fide Ltd. (A) During plantlets plantation (May 2022), (B) During fruits harvesting (end July 2022).

Table 2. List of descriptors employed for plant qualitative and quantitative traits.

Morphological descriptor	Abbrev.	Scale/Unit	Description
Growth habit	GHA	[1-7] >> *1=very upright, 3=upright, 5=intermediate, 7=prostrate*	
Leaf blade lobes	LBO	[1-7] >> *1=very weak, 3= weak, 5=intermediate, 7=strong,9=very strong*	
Anthocyanin distribution in plant	ADP	[1-7] >> *1=absent, 3=low, 5=intermediate, 7=high*	General anthocyanin distribution in apex, stem, calyx, prickles, leaf veins
Anthocyanin distribution in leaves	ADL	[1-7] >> *1=absent, 3=low, 5=intermediate, 7=high*	Anthocyanin distribution in leaf blade (intervein) as many times is different from the other tissues
Leaf prickliness	LPR	[0-9] >> *0=none, 1=very few (1-2), 3=few (3-5), 5=intermediate (5-10), 7= many (11-20), 9= very many (>20)*	3 representative fully expanded leaves.
Leaf hairiness	LHA	[0-5] >> *0=none, 1= low, 3=intermediate, 5=high*	3 representative fully expanded leaves.
Corolla color	CCO	[0-10] >> *0=yellow, 1=green, 2=greenish white, 3=white, 4=rose, 5=pink, 6=dark pink, 7=pale violet, 8= violet, 9=dark violet ,10=blue*	
Fruit load	FLO	[0-9] >> *0= none, 1=very low, 3=low, 5=intermediate, 7=high, 9=very high*	
Stem color	SCO	[1-5] >> *1=green, 3=greenish purple, 5=purple,*	Measured in 3 representative fully expanded leaves.
Petal color	PCO	[1-5] >> *1=green, 3=greenish purple, 5=purple.*	Measured in 3 representative fully expanded leaves.
Leaf blade width	LBW	Cm	Measured in 3 representative fully expanded leaves)
Leaf blade length	LBL	Cm	Measured in 3 representative fully expanded leaves)
Total plant height	TPH	Cm	Measured in the principal stem at the end of cropping period. Average of 2 plants per rep/accession

Table 3. List of Descriptors employed for fruit qualitative traits.

Morphological descriptor	Abbrev.	Scale/Unit	Description
Varietal type	VTY	[1-7] >> *1=long, 3=oval, 5=round, 7=striped.*	According to local description already existing
Predominant fruit color	PFC	[0-10] >> *0=dark green, 1=green, 2=milk white, 3=deep yellow, 4=fire red, 5= scarlet red, 6= lilac, 7= dark lilac, 8=purple.*	
Secondary fruit color	SFC	[0-10] >> *0=dark green, 1=green, 2=milk white, 3=deep yellow, 4=fire red, 5= scarlet red, 6= lilac, 7= dark lilac, 8= purple, 9=dark purple, 10=black.*	
Fruit color distribution	FCD	[1-7] >> *1=uniform, 3=mottled, 5=netted, 7=striped*	
Fruit undercalyx colour	FUC	[0-2] >> *0= absent, 1= intermediate, 2=present*	Presence of a lighter peel color edge next to calyx
Fruit glossiness	FGL	[1-3] >> *1=opaque, 2=intermediate, 3=bright peel color*	3 representative fruits /block/ accession
Fruit curvature	FCU	[0-9] >> *0=round, 1=no curvature, 3=slightly curved, 5=curved, 7=S shaped, 9= U shaped*	3 representative fruits /block/ accession
Fruit apex shape	FAS	[3-7] >> *3= protruding, 5=smooth, 7=depressed*	3 representative fruits /block/ accession
Position of the maximum diameter	PMD	[2-8] >> *3= about ¼ way from base to tip, 5= about ½ way from base to tip, 7= about ¾ way from base to tip*	3 representative fruits /block/ accession
Fruit cross section	FCS	[1-7] *1=circular, 3=elliptic, 5=smashed, 7=very irregular*	3 representative fruits /block/ accession
Presence of grooves on fruit	PGF	[1-3] >> *1=absent, 3=present*	
Presence of hole in fruit	PHF	[1-3] >> *1=absent, 3=present*	
Fruit end button size	FEB	[0-3] >> *0=none, 1=small, 2=intermediate, 3=large*	
Fruit shape	FSH	[1-9] >> *1=broader than long, 3=as long as broad, 5=Slightly longer than broad, 7=Twice as long as broad, 8=Three time as long as broad, 9=several times as long as broad*	
Presence of clorophyll on the pistil scar	PCP	[1-3] >> *1=absent, 3=present*	
Seed content	SEC	[1-3] >> *1=absent, 3=present*	

Table 4. List of descriptors employed for fruit qualitative and quantitative traits.

Morphological descriptor	Abbrev.	Scale/Unit	Description
Fruit weight	FWE	g	
Fruit length	FLE	Cm	
Fruit maximum diameter	FMD	Cm	
Peduncle length	PLE	Cm	
Fruit calyx prickliness	FCP	[0-9] >> 0=none, 1=Very few (<3), 3=Few (~5), 5=Intermediate (~10), 7=Many (~20), 9= Very many (>30)	Average of 3 values/block
Calyx fruit coverage	CFC	[1-5] >> 1= less than 10%, 2=10-20%, 3=20-30%, 4=30-40%, 5=50% and more	Average of 3 values/block
Locule number	LON	Count	Average of 3 values/block
Presence of a greenish ring next to the peel	PGP	[0-2] >> 0=no, 1= slight, 2=yes and markedly green	Average of 3 values/block
Average color of the flesh	ACF	[1-7] >> 1=white, 3=greenish, 5=green, 7=cream	Average of 3 values/block

Figure 2. Steps of morphological analysis (A) & (B) Fruit samples collection in the open field of Fidesan Fide Ltd. (Antalya – July 2022), (C) Fruit samples after collection.

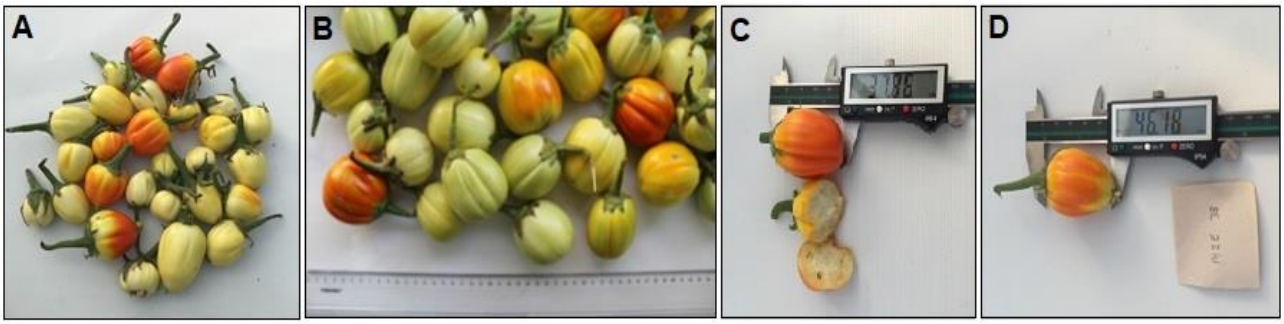

Figure 3. Fruit samples collected in greenhouse of BATEM Institute (January 2021) (A) Fruits presentation, (B) Fruits presentation next to a ruler, (C) Fruit height measurement with digital caliper and transverse section of fruit, (D) Fruit height measurement with digital caliper.

Figure 4. Fruit samples collected in Antalya – July 2022 (Fidesan Fide Ltd.).

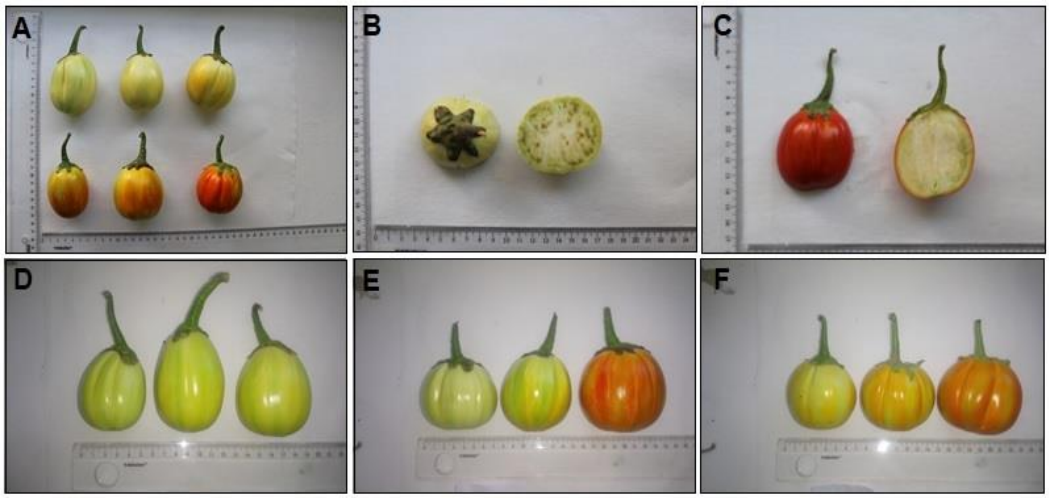

Figure 5. Fruit samples display (A) Fruits presentation, (B) Transverse section of fruit, (C) Longitudinal section of fruit (D) GKE7 fruit samples, (E) MZE49 fruit samples, (F) MZE47 fruit samples.

Table 5. Descriptive statistic summary for the studied quantitative variables in the greenhouse experiment.

Trait category	Descriptor abbreviations	Mean	STD	Range	
				Min	Max
Qualitative traits of plant	LBW	23.51	3.59	14.75	31.75
	LBL	32.63	3.41	24.00	40.50
	TPH	139.29	39.28	70.00	240.00
Quantitative traits of fruit	FWE	25.75	9.53	10.00	45.00
	FLE	4.01	0.77	2.60	5.57
	FMD	3.50	0.51	2.50	4.70
	PLE	3.61	0.61	2.25	4.90
	LON	5.85	1.37	3.00	9.00

Table 6. Correlation coefficient between descriptors in the greenhouse experiment.

Row	LBW	LBL	TPH	FWE	FLE	FMD	PLE	LON
LBW		0.612	0.026	0.249	0.018	0.293	-0.311	0.183
LBL			0.368	0.542	0.240	0.467	-0.031	0.077
TPH				0.339	0.221	0.281	0.157	0.109
FWE					0.801	0.937	0.588	0.336
FLE						0.741	0.616	0.262
FMD							0.554	0.423
PLE								0.198
LON								

LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number

accession GKE11) to 45 g (for accession MZE41), fruit length ranged from 2.6 cm (for accession MZE51) to 5.6 cm (for accession MZE53), fruit maximum diameter ranged from 2.5 cm (for accession MZE36) to 4.7 cm (for accession MZE41), peduncle length ranged from 2.3 cm (for accession MZE57) to 4.9 cm (for accessions MZE53 and MZE48), and locule number ranged from 3 (for accession GKE20) to 9 (for accession MZE53).

The statistical correlation test between all 8 studied quantitative traits showed corresponding correlation coefficients in Table 6. It was found that both positive and negative correlations prevail among morphological and agricultural valuable traits.

The correlation coefficient r values were all significant at $P < 0.05$. For instance, more positive significant correlation coefficients (values above 0.6) are listed as follow: fruit weight (FWE) was the most highly correlated with fruit maximum diameter (FMD) and fruit length (FLE) with r values of 0.937 and 0.801, respectively. Fruit length (FLE) was greatly correlated with fruit maximum diameter (FMD) and peduncle length (PLE) with r values of 0.741 and 0.616, respectively. Additionally, leaf blade width (LBW) was moderately correlated with leaf blade length (LBL) with r value of 0.612.

Results from the PCA based correlation matrix yielded principal components where the two first ones weighted 67.43% of the total variances with eigenvalues greater than 1 (Table 7).

Thus, the first principal component (PC1) obtained from the evaluation of different morphologic and agronomic valuable traits among *Solanum aethiopicum* gr. Gilo accessions expressed 45.90% of the total variance, and it was dependent on FWE, FMD, and FLE. Moreover, PC2

Table 7. Details on principal components analysis (greenhouse experiment).

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
LBW	0.15	0.64	0.26	-0.14	-0.02	0.65	0.20	-0.06
LBL	0.30	0.52	-0.24	-0.14	0.37	-0.57	0.27	0.16
TPH	0.22	0.10	-0.69	0.62	-0.13	0.26	0.00	0.01
FWE	0.50	-0.02	-0.02	-0.15	-0.03	-0.10	-0.32	-0.78
FLE	0.43	-0.23	0.01	-0.21	-0.65	-0.09	0.51	0.15
FMD	0.49	-0.01	0.12	-0.09	-0.02	0.08	-0.63	0.58
PLE	0.32	-0.50	-0.05	-0.10	0.64	0.34	0.32	0.04
LON	0.24	0.00	0.62	0.71	0.07	-0.20	0.14	-0.03
Eigenvalue	3.67	1.72	0.99	0.81	0.32	0.25	0.19	0.04
Percent	45.90	21.53	12.34	10.17	4.02	3.13	2.39	0.53
Cum %	45.90	67.43	79.77	89.94	93.95	97.09	99.47	100.00

PC: Principal component, LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number

contributed 21.53% of the total variability and was tied to LBW and LBL (Figure 6).

The phenotypic hierarchical cluster analysis was carried out to display the relationship structure among 57 accessions of *Solanum aethiopicum* gr. Gilo. Phenotypic similarity was calculated from 7 agro-morphological traits (quantitative traits) based on Ward aggregation distances. Due to the lack of fruits at maturity stage, 24 accessions were excluded from the hierarchical cluster analysis. Two major clusters were clearly identified. Thus, cluster A consisted of 18 accessions, and cluster B was slightly smaller, with only 15 accessions (Figure 7).

3.2. The open field experiment (II)

A summary of the descriptive statistics (means, standard deviations, and maximum and minimum values) for the open field experiment (2022) is shown in Table 8. Descriptive analyses made from quantitative traits data of plant and fruit displayed wide range of variations among all 55 accessions of *Solanum aethiopicum* gr. Gilo. For instance, the minimum leaf blade width was 10.0 cm (for accession GKE11) and the maximum was 18.0 cm (for accessions GKE7, GKE13 and MZE33), leaf blade length ranged from 17.5 cm (for accession GKE11) to 25.5 cm (for accession GKE20), and total plant height was scaled from 120.0 cm (for GKE5) to 160.0 cm (for MZE27).

Besides, we also noted extent changes from qualitative traits of fruit on studied accessions. As an illustration, fruit weight ranged from 16.7 g (for accession MZE43) to 91 g (for accession MZE49), fruit length ranged from 3.5 cm (for accession MZE43) to 9.1 cm (for accession GKE4), fruit maximum diameter ranged from 2.1 cm (for accession MZE34) to 8.8 cm (for accession GKE4), peduncle length ranged from 2.2 cm (for accessions MZE23 and MZE41) to 4.0 cm (for accession GKE3), and locule number ranged from 4 (for accessions MZE23 and MZE58) to 9 (for accessions GKE19 and MZE37).

The statistical correlation test between all 8 studied quantitative traits showed corresponding correlation coefficients in Table 9. It was found that both positive and negative correlations prevail among morphological and agricultural valuable traits. Correlation coefficient r values were all significant at $P < 0.05$. For instance, more positive significant correlation coefficients (values above 0.7) are listed as follow; fruit length (FLE) was the highly correlated with fruit maximum diameter (FMD) with r value of 0.853. Additionally, leaf blade width (LBW) was greatly correlated with leaf blade length (LBL) with r value of 0.710.

Results from PCA based correlation matrix yielded principal components where three first ones weighted 65.65% of the total variances with eigenvalues more than 1 (Table 10).

The results of principal components analysis are shown in Figure 8. The first two components are displayed.

The first principal component (PC1) was obtained from the evaluation of different morphologic and agronomic valuable traits among *Solanum aethiopicum* gr. Gilo accessions expressed 28.65% of the total variance and it was dependent on FMD and FLE. PC2 contributed 23.60% of the total variability and was tied to LBW and LBL. The third component (PC3) accounted for 13.40% of total variance and it is more related to PLE and TPH.

The phenotypic hierarchical cluster analysis was carried out to display the relationship structure among 55 accessions of *Solanum aethiopicum* gr. Gilo. Phenotypic similarity was calculated from 7 agro-morphological traits (quantitative traits) based on Ward aggregation distances (Figure 9).

Five accessions were excluded in the hierarchical clustering analysis due to the lack of fruits at maturity stage. Two major clusters were clearly identified. Thus, cluster A consisted of 26 accessions, while cluster B was slightly smaller, with only 24 accessions.

3.3. General remarks

The summary of descriptive statistics of both experiments of agro-morphological characterization of different accessions of *Solanum aethiopicum* gr. Gilo implies that there are significant differences between accessions. Lester and Daunay (2003) previously reported on this finding. These

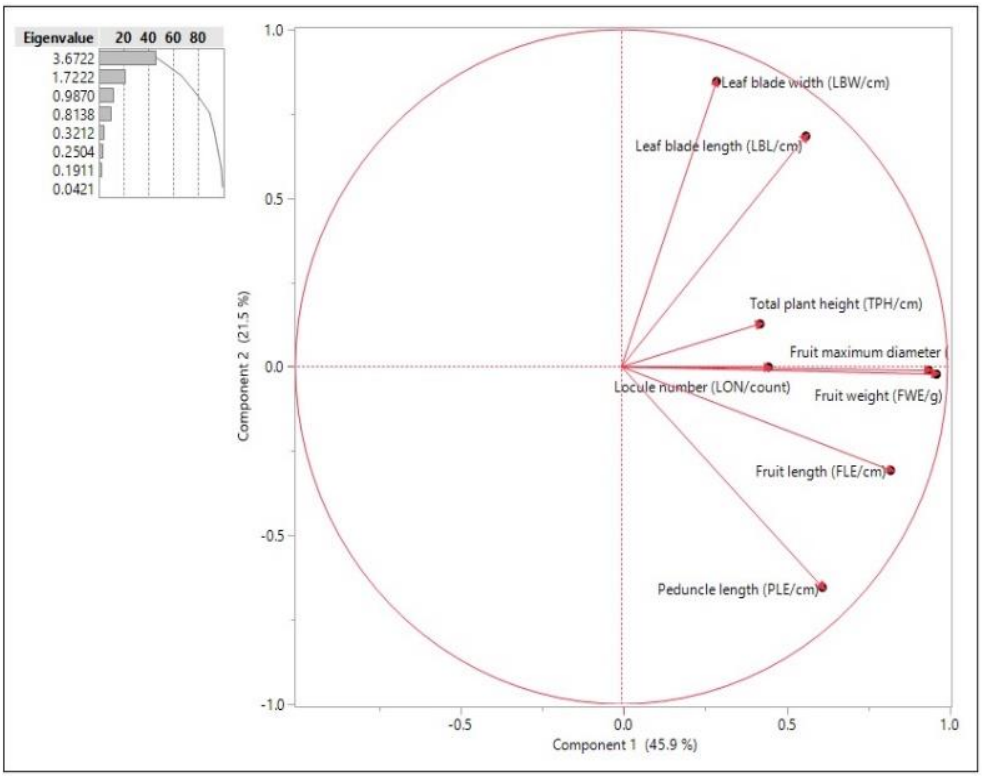

Figure 6. Summary plot on variables based Principal Components in the greenhouse experiment.

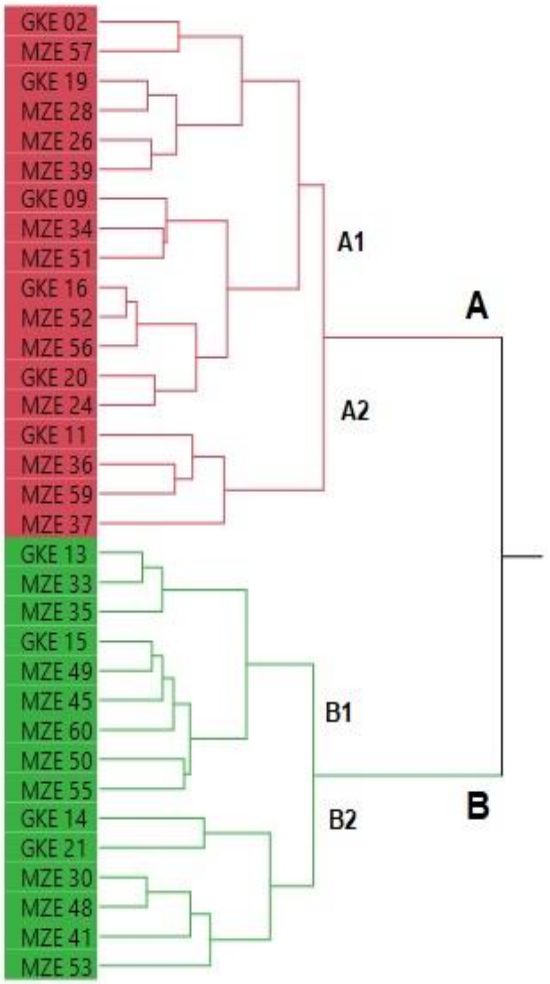

Figure 7. Hierarchical clustering analysis (HCA) dendrogram derived from greenhouse experiment.

Table 8. Descriptive statistic summary for the studied quantitative variables in the open field experiment.

Trait category	Descriptor abbreviations	Mean	STD	Range	
				Min	Max
Qualitative traits of plant	LBW	14.71	1.84	10.00	18.00
	LBL	21.45	2.14	17.50	25.50
	TPH	142.60	8.54	120.00	160.00
Quantitative traits of fruit	FWE	52.97	16.92	16.67	91.00
	FLE	5.38	0.94	3.54	9.15
	FMD	4.78	0.98	2.15	8.82
	PLE	2.97	0.42	2.17	4.00
	LON	6.16	1.18	4.00	9.00

LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number

Table 9. Correlation Coefficient between descriptors in the open field experiment.

Row	LBW	LBL	TPH	FWE	FLE	FMD	PLE	LON
LBW		0.710	0.155	-0.084	0.092	0.025	-0.164	-0.189
LBL			0.172	-0.178	0.032	-0.043	-0.105	-0.001
TPH				-0.023	0.215	0.177	0.032	0.008
FWE					0.282	0.341	-0.019	0.335
FLE						0.853	0.037	0.220
FMD							-0.115	0.355
PLE								0.011
LON								

LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number

Table 10. Details on principal components analysis (open field experiment).

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
LBW	-0.03	0.65	-0.11	0.15	-0.21	0.21	0.68	-0.05
LBL	-0.05	0.63	-0.08	0.37	-0.01	-0.13	-0.65	0.11
TPH	0.16	0.28	0.55	-0.19	0.72	0.21	0.03	0.00
FWE	0.39	-0.17	-0.28	0.29	0.03	0.80	-0.16	0.01
FLE	0.57	0.14	0.18	-0.21	-0.33	-0.11	-0.14	-0.66
FMD	0.60	0.08	0.01	-0.23	-0.18	-0.18	0.06	0.71
PLE	-0.03	-0.19	0.73	0.54	-0.35	0.06	0.06	0.13
LON	0.37	-0.14	-0.21	0.58	0.41	-0.46	0.25	-0.14
Eigenvalue	2.29	1.89	1.07	0.91	0.80	0.69	0.24	0.12
Percent	28.65	23.60	13.40	11.36	9.95	8.63	2.96	1.45
Cum %	28.65	52.25	65.65	77.01	86.95	95.58	98.55	100.00

PC: Principal component, LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number

researchers stated that the fruits and leaves of *Solanum aethiopicum* vary in shape and size within and between cultivar groups (Gilo, Kumba, Shum, and Acelatum).

When the mean values of different quantitative agro-morphological traits were compared, it was observed that there was a significant difference between experiments (or between greenhouse and open field cultivation) in terms of plant development and yield features (Figure 10).

The greenhouse experiment produced significantly larger leaves (LBW and LBL) than the open-field experiment. In contrast, yield characteristics (FWE, FLE, and FMD) were better in the open field experiment than in the greenhouse experiment. Although the obtained mean values of fruit weight in both experiments (25.75 and 52.97 g) were lower than the mean value reported by Prohens et al. (2005) on the morphological diversity assessment between three accessions of *Solanum aethiopicum* gr. Gilo and other eggplants, which was 74.30 g.

Substantial variations between experiments can be identified by analyzing other morphological traits, particularly qualitative traits (Table 11). Some of these variations include the distribution of anthocyanin in plants and leaves (ADP and ADL).

For instance, plants and leaves in greenhouse experiment had higher anthocyanin levels than plants and leaves in open field experiment. Similarly, in greenhouse experiment, the leaves had more prickles and were hairier than in open field experiment.

Furthermore, in the greenhouse experiment, eggplants had a higher fruit load (FLO) than in the open field experiment. However, the yield characteristics (quantitative traits) change in favor of the open field experiment at the maturity stage. The high seed content (SEC) observed in the open field experiment confirms the above. In summary, greenhouse-grown eggplants have better overall plant qualitative traits, but their fruit yield qualities are not substantial. The findings of multiple correlation analysis were used to compare the two

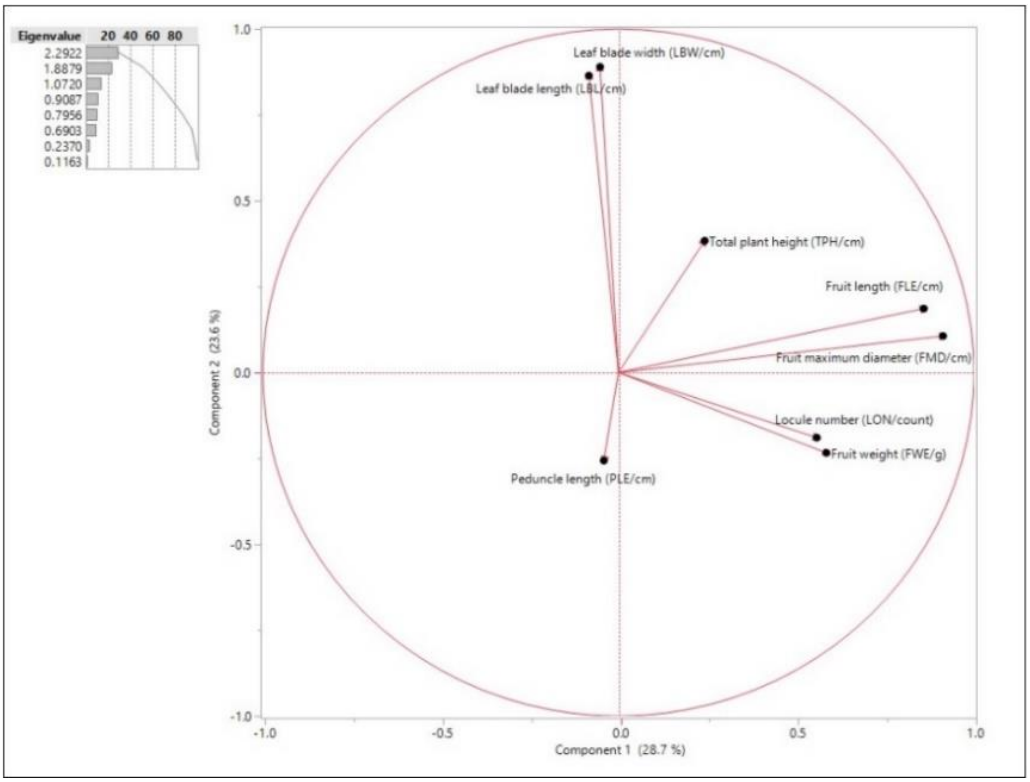

Figure 8. Summary plot on variables based Principal Components in the open field experiment.

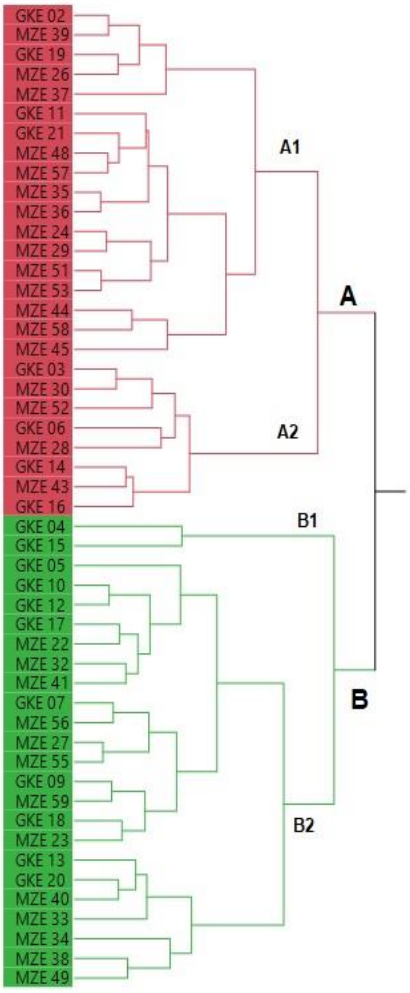

Figure 9. Hierarchical clustering analysis (HCA) dendrogram derived from open field experiment.

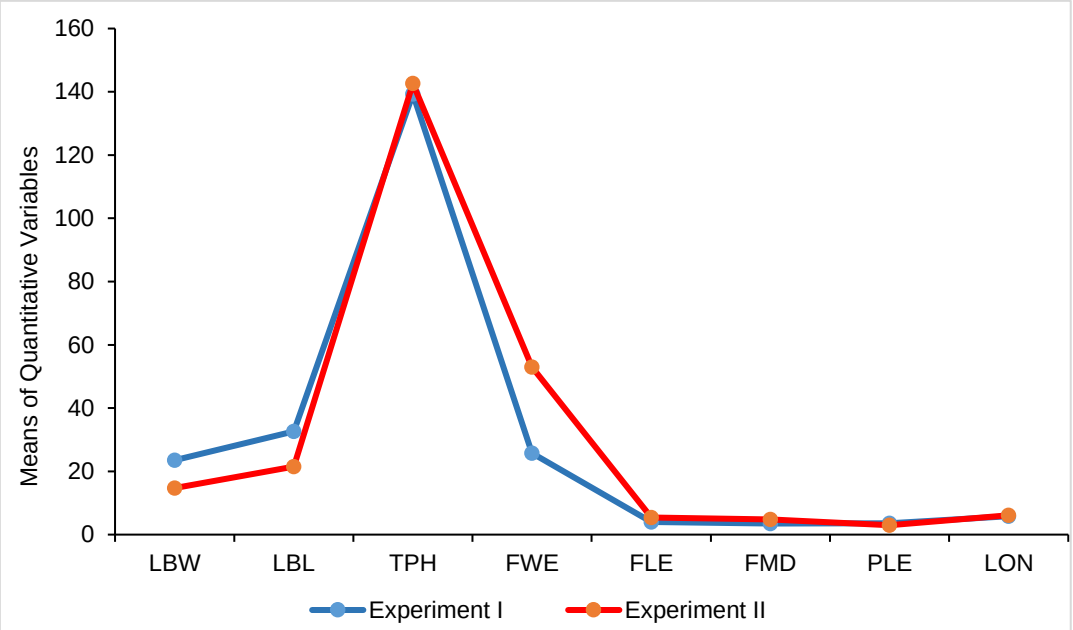

Figure 10. Means comparison of experiments I (greenhouse) and II (open field) (LBW: Leaf blade width, LBL: Leaf blade length, TPH: Total plant height, FWE: Fruit weight, FLE: Fruit length, FMD: Fruit maximum diameter, PLE: Peduncle length, LON: Locule number).

Table 11 Range and mean of qualitative traits between experiments.

	Experiment I		Experiment II	
Descriptors	Range (Min-Max)	Mean	Range (Min-Max)	Mean
GHA	1-7	4.33	3-7	3.32
LBO	3-9	6.15	5-7	6.36
ADP	1-7	5.06	1-5	3.16
ADL	1-7	5.00	1-5	3.08
LPR	0-5	1.03	0-0	0.0.
LHA	3-7	4.57	0-3	0.06
CCO	3-3	3.00	3-3	3.00
FLO	1-5	2.09	0-7	1.76
SCO	1-5	3.36	1-3	1.08
PCO	1-3	1.96	1-5	1.15
VTY	3-5	3.48	3-5	3.28
PFC	1-1	1.00	1-5	4.12
SFC	1-1	1.00	0-5	4.04
FCD	1-1	1.00	1-7	1.64
FUC	0-0	0.00	0-0	0.00
FGL	3-3	3.00	3-3	3.00
FCU	1-1	1.00	0-1	0.54
FAS	3-5	3.66	3-7	4.88
PMD	5-7	6.15	5-5	5.00
FCS	1-7	5.12	1-7	3.60
PGF	3-3	3.00	3-3	3.00
PHF	1-3	2.75	1-3	2.76
FEB	1-5	3.96	1-3	1.60
FSH	3-5	4.51	1-5	4.84
PCP	1-3	2.09	1-3	1.20
SEC	1-3	1.06	1-3	2.92
FCP	0-1	0.06	0-0	0.00
PGP	0-1	0.06	0-1	0.22
ACF	3-7	6.63	1-7	2.72

GHA: Growth habit, LBO: Leaf blade lobes, ADP: Anthocyanin distribution in plant, ADL: Anthocyanin distribution in leaves, LPR: Leaf prickliness, LHA: Leaf hairness, CCO: Corolla color, FLO: Fruit load, SCO: Stem color, PCO: Petal color, VTY: Varietal type, PFC: Predominant fruit color, SFC: Secondary fruit color, FCD: Fruit color distribution, FUC: Fruit undercalyx color, FGL: Fruit glossiness, FCU: Fruit curvature, FAS: Fruit apex shape, PMD: Position of the maximum diameter, FCS: Fruit cross-section, PGF: Presence of grooves on fruit, PHF: Presence of hole in fruit, FEB: Fruit end button size, FSH: Fruit shape, PCP: Presence of chlorophyll on the pistil scar, SEC: Seed content, FCP: Fruit calyx prickliness, PGP: Presence of a greenish ring next to the peel, ACF: Average color of the flesh

experiments once more (Figure 11). It was discovered that highly correlated variables represented fruit quantitative traits. The greenhouse experiment had fruit weight (FWE) highly correlated to fruit maximum diameter (FMD) (r=0.937), as well as fruit weight (FWE) highly correlated to fruit length (FLE) (r=0.801), and the open field experiment had fruit length (FLE) highly correlated to fruit maximum diameter (FMD) (r=0.853). This means that the fruit descriptor variables were more dependent upon one another than the plant descriptor variables.

The two phenograms or hierarchical clustering analyses (HCA) generated using eight morphological descriptors based on the Ward aggregation distance clustering method clearly displayed the phonetic relationship among the accessions based on similarity and relatedness of eggplants. For the greenhouse experiment, it separated the 33 accessions (from 57 accessions originally assessed) into two major clusters (cluster A and cluster B) and it was found that each cluster accommodated accessions from both Gakenke and Musanze districts. A thorough examination of the phenotypic clustering results (greenhouse experiment) and morphological traits' mean performances (summary on descriptive statistics on quantitative traits-greenhouse experiment), have revealed that Cluster "B" does accommodate all superior elements in terms of fruit quantitative traits such as; fruit weight (FWE-MZE41), fruit length (FLE-MZE53), fruit maximum diameter (FMD-MZE41), peduncle length (PLE-MZE53 and MZE48) and locule number (LON, MZE53) as well as plant quantitative traits such as; leaf blade width (LBW-MZE53), leaf blade length (LBL-GKE14) and total plant height (TPH-GKE21). Besides, cluster "A" does also contain superior accessions in regard to LBW, LBLT and LPH (qualitative traits of the plant). These results made accessions from this cluster promising donor parents for multiple traits.

For the open field experiment, it separated the 50 accessions (from 55 accessions originally assessed) into two major clusters (cluster A and cluster B) and it was found that each cluster accommodated accessions from both Gakenke and Musanze districts. A thorough examination on the phenotypic clustering results (open field experiment) and morphological traits' mean performances (summary on descriptive statistics on quantitative traits-open field experiment), have revealed that Cluster "B" does accommodate all superior elements in terms of fruit quantitative traits such as; fruit weight (FWE-MZE49), fruit length (FLE-GKE4), fruit maximum diameter (FMD-GKE4), as well as plant quantitative traits such as; leaf blade width (LBW-GKE7, GKE13, and MZE33), leaf blade length (LBL-GKE20) and total plant height (TPH-MZE27). Although, cluster "A" does also contain superior accessions in regard to fruit qualitative traits of the plant like peduncle length (PLE, GKE3) and locule number (LON-GKE19 and MZE37). These results made accessions from mainly cluster B promising donor parents for multiple traits.

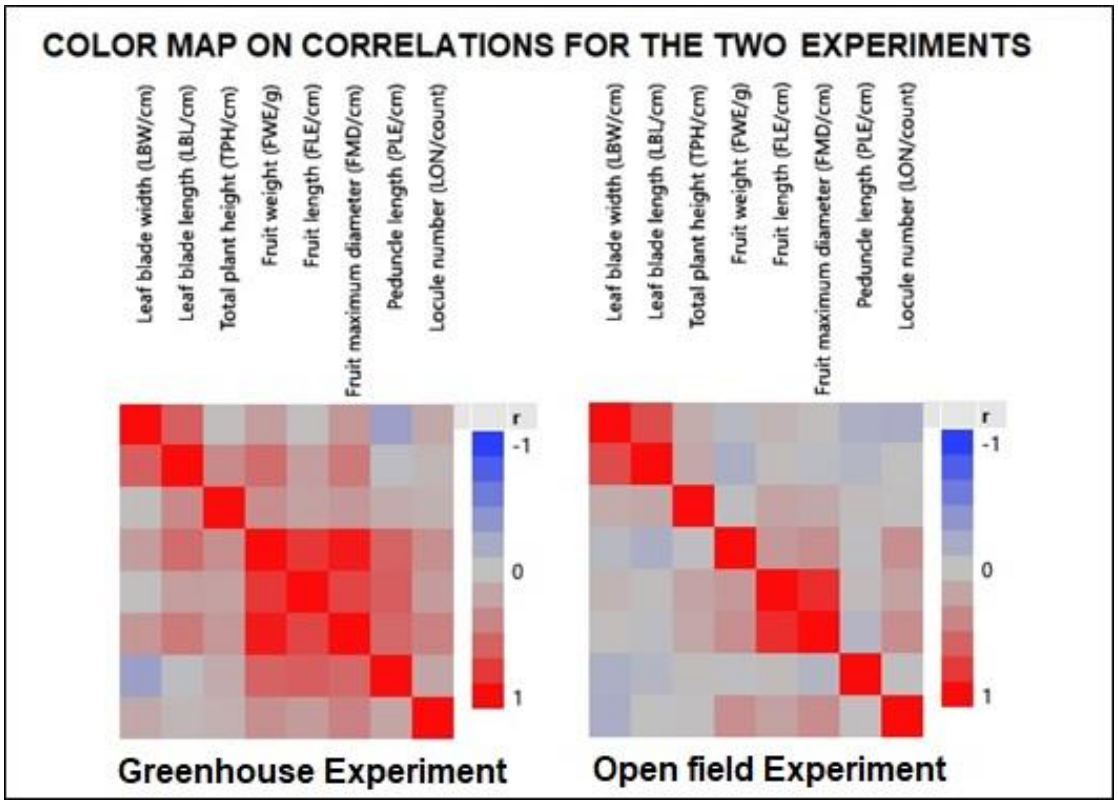

Figure 11. Comparison of multiple correlation analysis.

Furthermore, the evidence based on the PCA correlation matrix in greenhouse experiment has assured the significance of fruit quantitative variables (FWE, FMD, and FLE) in the first principal component (PC1), which explained 45.90% of the total variance within the studied germplasm. Similarly, in open field experiment, fruit quantitative variables (FMD and FLE) were substantial in the first principal component (PC1), which explained 45.90% of the total variance within the studied germplasm.

This means that the overall differences observed in *Solanum aethiopicum* gr. Gilo germplasm both in greenhouse and in open field experiments were due mainly to fruit quantitative traits. In other words, the chosen morphological descriptors, particularly those related to yield and fruit quality, were conclusive for eggplant germplasm characterization. The phenotypic results provide new awareness on the agronomic behavior of several accessions. For future breeding programs, accessions with high average fruit weight, fruit maximum diameter, and fruit length could be taken into consideration.

In brief, this high diversity is often based on high discriminatory fruit traits of *Solanum aethiopicum* and has been highlighted in diversity studies by Adeniji et al. (2012), Plazas et al. (2014), Sakhanokho et al. (2014), and Bationo-Kando et al. (2015). It was also reported that different fruit sizes and shapes exist among individuals of *Solanum aethiopicum* gr. Gilo (Taher et al., 2017). Our results show the existence of an extensive phenotypic diversity in *Solanum aethiopicum* gr. Gilo which is in agreement with similar results by Kouassi et al. (2014). Observations from fruit traits reaffirm that the used germplasm of *Solanum aethiopicum* belongs to the Gilo group and it is more particularly similar to one of three sub-groups reported by the same authors in Côte d'Ivoire and which is known as "N'Drowa". Thus, it was found that the N'Drowa subgroup has larger leaves and larger fruits. Several scholars pointed out the high morphological diversity of scarlet eggplant on the African continent, as well as the existence of large germplasm collections made of several landraces in countries such as Burkina Faso, Cameroon, Côte d'Ivoire, Ethiopia, Gabon, Ghana, Kenya, Nigeria, Senegal, Tanzania, Uganda, Zambia, Zimbabwe, and also Rwanda. Furthermore, those listed countries are seen as domestication centers of *Solanum aethiopicum* (Sseremba, 2019).

4. Conclusion

Landraces are crucial in contemporary plant breeding programs due to their extensive genetic diversity compared to modern varieties. As a direct consequence, they may be useful to expand the genetic base of modern cultivars. In this study, the genetic and phenotypic diversity of the largest germplasm collection of *Solanum aethiopicum* gr. Gilo accessions from Rwanda was assessed using the most recent molecular markers, the iPBS retrotransposon markers system for genetic diversity, and a couple of descriptors for eggplant morphological characterization.

Similarly, phenotypic diversity analysis revealed a high level of diversity among *Solanum aethiopicum* gr. Gilo accessions. Multiple correlations disclosed that plant and fruit-related descriptors diverge enough to distinguish between germplasm populations. Fruit variables/descriptors, for instance, were more interdependent than plant variables/descriptors. Furthermore, phenotypic (hierarchical) clustering shows that clusters were formed primarily on the basis of fruit quantitative traits rather than plant quantitative traits. This means that fruit quantitative traits are critical for phenotypic characterization of *Solanum aethiopicum* gr. Gilo eggplant.

The information produced by deep characterization of base collection could be useful in future germplasm characterization. Thus, the conservation of heritage of *Solanum aethiopicum* gr. Gilo may be provided for future generations.

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References

Adeniji, O.T., Kusolwa, P.M., & Reuben, S.O.W.M. (2012). Genetic diversity among accessions of *Solanum aethiopicum* L. groups based on morpho-agronomic traits. *Plant Genetic Resources,* 10:177-185.

Bationo-Kando, P., Sawadogo, B., Nanema, K.R., Kiebre, Z., Sawadogo, M., & Zongo, J.D. (2015). Characterization of *Solanum aethiopicum* (Kumba group) in Burkina Faso. *International Journal of Science and Nature,* 6:169–176.

Boyaci, H.F., Prohens, J., Unlu, A., Gumrukcu, E., Oten, M., & Plazas, M. (2020). Association of heterotic groups with morphological relationships and general combining ability in eggplant. *Agriculture,* 10:203.

Caguiat, X.G.I., & Hautea, D.M., (2014). Genetic diversity analysis of eggplant (*Solanum melongena* L.) and related wild species in the Philippines using morphological and SSR markers. *SABRAO Journal of Breeding and Genetics,* 46:183–201.

Context Network (2016). Rwanda early generation seed study: Country report. https://reliefweb.int/report/rwanda/rwanda-early-generation-seed-study-country-report. Accessed date: September 7, 2022.

Dijkxhoorn, Y., Saavedra Gonzalez, Y., & Judge, L.O. (2016). Horticulture and floriculture in Rwanda: Identification of focus areas for sector development. https://www.wur.nl/en/Publication-details.htm?publica

tion Id=publication-way-353134333031. Accessed date: September 7, 2022.

Dusengemungu, L., Igiraneza, C., & Uwimbabazi, S. (2019). Plant biotechnology: A key tool to improve crop production in Rwanda. *African Journal of Biotechnology,* 18:68–76.

Eletta, O.A.A., Orimolade, B.O., Oluwaniyi, O.O., & Dosumu, O.O. (2017). Evaluation of proximate and antioxidant activities of Ethiopian eggplant (*Solanum aethiopicum* L) and gboma Eggplant (*Solanum macrocarpon* L). *Journal of Applied Science and Environmental Management,* 21:967–972.

Foo, P.C., Lee, Z.H., Chin, C.K., Subramaniam, S., & Chew, B.L. (2018). Shoot Induction in white eggplant (*Solanum melongena* L. Cv. Bulat Putih) using 6-Benzylaminopurine and Kinetin. *Tropical Life Sciences Research,* 29:119–129.

Gapusi, R.J., Isibo, T., Nyirigira, R.A., Gashamura, R.F., Sirikari, S., Uwimana, J.D.A., Byamushana, C., Bizeye, B., & Hakizimana, P. (2013). The state of plant genetic resources for food and agriculture in Rwanda, Country Report. Rwanda Agriculture and Animal Resources Development Board, Kigali, Rwanda.

Gramazio, P., Blanca, J., Ziarsolo, P., Herraiz, F.J., Plazas, M., Prohens, J., & Vilanova, S. (2016). Transcriptome analysis and molecular marker discovery in *Solanum incanum* and *S. aethiopicum*, two close relatives of the brinjal eggplant (*Solanum melongena*) with interest for breeding. *BMC Genomics,* 17: 300.

Haliński, Ł.P., Samuels, J., & Stepnowski, P. (2017). Multivariate analysis as a key tool in chemotaxonomy of brinjal eggplant, African eggplants and wild related species. *Phytochemistry,* 144:87–97.

Isshiki, S., Uchiyama, T., Tashiro, Y., & Miyazaki, S. (1998). RFLP analysis of a PCR amplified region of chloroplast DNA in eggplant and related *Solanum* species. *Euphytica,* 102:295–299.

Kamga, R.T., Kouamé, C., Atangana, A.R., Abdulai, M.S., & Tenkouano, A. (2015). Characterization of African eggplant accessions for morphological and yield parameters in the bimodal rainfall agroecology of Cameroon. *Acta Horticulturae,* 1102:109–120.

Kamga, R.T., Kouamé, C., Atangana, A.R., Chagomoka, T., & Ndango, R. (2013). Nutritional evaluation of five African indigenous vegetables. *Journal of Horticultural Research,* 21:99-106.

Kansiime, M.K., Ochieng, J., Kessy, R., Karanja, D., Romney, D., & Afari-Sefa, V. (2018). Changing knowledge and perceptions of African indigenous vegetables: The role of community-based nutritional outreach. *Development in Practice,* 28: 480–493.

Knapp, S., Aubriot, X., & Prohens, J. (2019). Eggplant (*Solanum melongena* L.): Taxonomy and Relationships. In M. Chapman (Ed), *The Eggplant Genome. Compendium of Plant Genomes*. Springer, Cham, pp 11–22.

Knapp, S., Vorontsova, M.S., & Prohens, J. (2013). Wild relatives of the eggplant (*Solanum melongena* L.: Solanaceae): New understanding of species names in a complex group. *PLoS One,* 8:e57039.

Kouassi, A., Béli-Sika, E., Tian-Bi, T., Alla-N'Nan, O., Kouassi, A., N'Zi, J.C., N'Guetta, A.S.P., & Tio-Touré, B. (2014). Identification of three distinct eggplant subgroups within the *Solanum aethiopicum* gilo group from Côte d'Ivoire by morpho-agronomic characterization. *Agriculture,* 4:260–273.

Larochelle, C., & Alwang, J.R. (2014). Impacts of improved bean varieties on food security in Rwanda In: *2014 Annual meeting - Agricultural and Applied Economics Association (AAEA) Conferences-2014 Annual Meeting*. Minneapolis, pp 1–27.

Lester, R.N., & Daunay, M.C. (2003). Diversity of African vegetable Solanum species and its implications for a better understanding of plant domestication. In H. Knüpffer, & J. Ochsmann (Eds), *Proceedings of a symposium dedicated to the 100th birthday of Rudolf Mansfeld (8-9 October 2001)*. Zentralstelle für Agrardokumentation und -information (ZADI), Gatersleben, pp 136–152.

Mibei, E.K., Owino, W., Ambuko, J., Giovannoni, J.J., & Onyango, A.N. (2018). Metabolomic analyses to evaluate the effect of drought stress on selected African Eggplant accessions. *Journal of the Science of Food and Agriculture,* 98:205–216.

Mujuju, C. (2018). *Identifying Leading Seed Companies in Western and Central Africa*. Access to Seeds Foundation, Harare, Zimbabwe.

Osei, M.K., Banful, B., Osei, C.K., & Oluoch, M.O. (2010). Characterization of African eggplant for morphological characteristics. *Journal of Agricultural Science and Technology,* 4:33–37.

Plazas, M., Andújar, I., Vilanova, S., Gramazio, P., Javier Herraiz, F., & Prohens, J. (2014). Conventional and phenomics characterization provides insight into the diversity and relationships of hypervariable scarlet (*Solanum aethiopicum* L.) and gboma (*S. macrocarpon* L.) eggplant complexes. *Frontiers in Plant Science,* 5:318.

Prasad, P.V.V., Hijmans, R.J., Pierzynski, G.M., & Middendorf, J.B. (2016). Climate Smart Agriculture and Sustainable Intensification: Assessment and Priority Setting for Rwanda. Feed the Future Sustainable Intensification Innovation Lab (Kansas State University), Manhattan, KS.

Prohens, J., Blanca, J., & Nuez, F. (2005). Morphological and molecular variation in a collection of eggplants from a secondary center of diversity: Implications for conservation and breeding. *Journal of the American Society for Horticultural Science,* 130:54–63.

Sakhanokho, H.F., Islam-Faridi, M.N., Blythe, E.K., Smith, B J., Rajasekaran, K., & Majid, M.A. (2014). Morphological and cytomolecular assessment of intraspecific variability in Scarlet eggplant (*Solanum aethiopicum* L.). *Journal of Crop Improvement,* 28:437–453.

Schreinemachers, P., Sequeros, T., & Lukumay, P.J. (2017). International research on vegetable improvement in East and Southern Africa: Adoption, impact, and returns. *Agricultural Economics,* 48:707–717.

Shimira, F., Afloukou, F., & Maniriho, F. (2020). A review on challenges and prospects of potato (*Solanum tuberosum*) production systems in Rwanda. *Journal of Horticulture and Postharvest Research,* 3:97–112.

Shimira, F., Boyaci, H.F., Çilesiz, Y., Nadeem, M.A., Baloch, F.S., & Taşkin, H. (2021). Exploring the genetic diversity and population structure of scarlet eggplant germplasm from Rwanda through iPBS-retrotransposon markers. *Molecular Biology Reports,* 48:6323-6333.

Song, B., Song, Y., Fu, Y., Kizito, E. B., Kamenya, S.N., Kabod, P.N., Liu, H., Muthemba, S., Kariba, R., Njuguna, J., Maina, S., Stomeo, F., Djikeng, A., Hendre, P.S., Chen, X., Chen, W., Li, X., Sun, W., Wang, S., Cheng, S., Muchugi, A., Jamnadass, R., Shapiro, H.Y., Van Deynze, A., Yang, H., Wang, J., Xu, X., Odeny, D.A., & Liu, X. (2019). Draft genome

sequence of *Solanum aethiopicum* provides insights into disease resistance, drought tolerance, and the evolution of the genome. *GigaScience,* 8:giz115.

Sseremba, G. (2019). *Genetic diversity and breeding of Solanum aethiopicum shum group for drought tolerance*. Dissertation, University of Ghana.

Taher, D., Solberg, S.Ø., Prohens, J., Chou, Y.Y., Rakha, M., & Wu, T.H. (2017). World vegetable center eggplant collection : Origin, composition, seed dissemination and utilization in Breeding. *Frontiers in Plant Science,* 8:1484.

Uwamahoro, F., Yuen, J., Berlin, A., Bucagu, C., & Bylund, H. (2020). *Ralstonia solanacearum* causing potato bacterial wilt: Host range and cultivars' susceptibility in Rwanda. *Plant Pathology,* 69:559–568.

Van Dijk, N., & Elings, A. (2014). *Horticulture in Rwanda: Possibilities for Further Development*. BoP Innovation Center – 28 (Wageningen University), Wageningen.

Weller, S.C., Van Wyk, E., & Simon, J.E. (2015). Sustainable production for more resilient food production systems: Case study of African indigenous vegetables in eastern Africa. *Acta Horticulturae,* 1102:289–297.

Zhuang, Y., Zhou, X., & Wang, S. (2012). Genetic diversity of NBS-LRR class disease-resistance gene analogs in cultivated and wild eggplants. *Plant Systematics and Evolution,* 298:1399–1406.

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RESEARCH PAPER

The Effect of Hydrodistillation Times and Cold Pressing on Yield and Composition of Sweet Orange (*Citrus sinensis*) Peel Essential Oil

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Orange

Abstract

Within the scope of the study, the effect of hydro-distillation times on *Citrus sinensis* (Navelina) fresh peel essential oil composition was investigated. For this purpose, five different distillation times (10, 15, 30, 60, and 120 min.) were evaluated. Research findings showed that the distillation time was not effective on the orange essential oil composition. It was determined that the most important components of *C.sinensis* peel essential oil were limonene (96.52-96.61%) and myrcene (2.03-2.06%). In addition, hydrodistillation (HD) and cold press (CP) essential oils were compared in terms of yield and some physical and chemical properties. In terms of oil yield and optical activity, the values of the oil obtained by hydrodistillation method were higher than those obtained by cold press, and the refractive index and density values were found to be lower. In terms of component ratios, it was seen that there was no significant difference between the two methods.

1. Introduction

The most important reason why the orange has a significant share in the world citrus production is that it is the most preferred citrus type, especially by the fruit juice industry. According to USDA data, a total of 49 million tons of oranges were produced in the world as of the 2020/21 season. Türkiye has some advantages in citrus cultivation in terms of geographical location and climatic conditions. Türkiye ranks 8th in world orange production with a production amount of 1.4 million tons annually. Most of orange production (85%) in Türkiye is carried out in the Mediterranean Region (Aygören, 2021).

Orange is the most widely consumed citrus fruit and accounts for 60% of the world's citrus production. During the processing of the juice, a large amount of by-products is obtained, mainly the peels, representing about 45% of the total mass. Studies have pointed out that orange peel waste may cause many environmental problems, especially water pollution (Hilali et al., 2019).

The consciousness of consumers about nutrition and health has led the food industry to develop food and food supplements enriched with bioactive compounds. Additionally, in order to protect the environment and open up a more efficient use area, the new trend consists of recycling valuable compounds found in industrial food processing wastes (Angiolillo et al., 2015).

The peels form about 25% of the citrus weight. The quality of essential oil obtained from citrus peel largely depends on many factors such as origin, soil type, climate, and citrus variety, but the way the fruit is processed also has a significant impact (Ferhat et al., 2016). Essential oils obtained from citrus peel consist of terpenes, sesquiterpenes, higher alcohols, aldehydes, and esters (Shan, 2016). As reported in the studies, the chemical composition of orange peel essential oil mainly consists of limonene (86%-97%), which is a hydrocarbon compound (Hosni et al., 2010; Gölükcü et al., 2015; Ferhat et al., 2016; Manaila et al., 2016; Sawi et al., 2019). The fact that limonene is one of the most

common compounds found in the essential oils of aromatic plants can be attributed to its leading role in the biosynthesis of other monoterpenes and its protective role against herbivores. Limonene is widely used as a flavor and fragrance additive in consumer products such as perfumes and beverages (Erasto and Viljoen, 2008). In addition, limonene is an important natural compound with a wide range of uses due to its antimicrobial, antioxidant, anti-inflammatory, antinociceptive, anticancer, and insecticidal effects (Karr and Coats, 1988; Erasto and Viljoen, 2008; Vieira et al., 2018; Ngan et al., 2020; Maurya et al., 2021).

It is stated that in the production of essential oil from some medicinal and aromatic plants, products with different compositions can be obtained depending on the distillation time (Zheljazkov et al., 2013; Gölükcü et al., 2018; Durazzini et al., 2019; Anastasopoulou et al., 2020). Within the scope of the research, the effect of five different hydrodistillation periods on the yield and composition of sweet orange peel essential oil was investigated. In addition, the essential oil compositions obtained by hydrodistillation and cold press methods were compared in the study.

2. Material and Methods

2.1. Plant material

Fresh *Citrus sinensis* Navelina orange peels were used as material in the study. The samples were taken from 20-25-year-old orange trees in Aksu location of the Batı Akdeniz Agricultural Research Institute (BATEM) on December 23, 2021. After the samples were harvested, they were sent to the BATEM Medicinal Aromatic Plants Center Laboratory for analysis. First of all, the fruit weight and peel ratio of the fruits were determined. For this purpose, 18 fruits were used for each replication.

2.2. Hydrodistillation (HD) process

The essential oil amounts of the samples were determined by the hydrodistillation method in the clevenger device (TSE, 2011). For this purpose, 50 g of fresh fruit peel was crushed with 150 ml of distilled water in a blender (Waring 8011ES, USA) and then distilled in a clevenger apparatus (Isotex, Türkiye). Determined distillation times were given considering the boiling point. The oil yield obtained in 120 minutes was accepted as 100% and the oil yield was calculated based on 100 g of fruit peel.

2.3. Cold press process

The essential oil was obtained by cold pressing according to Kırbaşlar et al. (2001). For this purpose, first of all, the flavedo parts of the fruit peels containing essential oil were grated with a hand-held grater. The resulting graters were subjected to manual pressing. The water:oil emulsion obtained as a result of pressing was centrifuged at 12000 rpm for 20 minutes in an ultracentrifuge at 20°C. The essential oils collected in the upper phase were taken into eppendorf tubes and analyzed.

2.4. GC/MS-FID analysis

Component analysis of orange peel essential oils obtained as a result of different distillation times for hydrodistillation and cold press was determined using a gas chromatography (Agilent 7890A) mass detector (Agilent 5975C) device (GC/MS-FID). Analyzes were carried out with reference to the method used by Özek et al. (2010). First of all, orange peel essential oil samples were diluted with hexane at a ratio of 1:50. In the chromatographic analysis, a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm) was used as the column, and helium gas at a flow rate of 0.8 mL min^{-1} was used as the carrier gas. The injection block temperature was set to 250°C. The column temperature program was adjusted as 60°C (10 minutes), from 60°C to 220°C at 4°C/minute and 220°C (10 minutes). The data of Wiley7n, Oil Adams, and Nist05 libraries were used to identify the essential oil components of the samples. The percentage of each essential oil compoenents was determined by using flame ionization dedector values.

2.5. Physical analysis

Relative density (TS, 2012a), refractive index (TS, 2009), and optical activity (TS, 2012b) analyzes were made according to the Turkish Standard.

2.6. Statistical analysis

The research was carried out in a randomized plot design with three replications. Essential oil component analyzes were performed as two injections per repetition. The data obtained as a result of the study were subjected to the Duncan Multiple Comparison test using the SAS package program. Obtained statistical data are presented as mean±standard error.

3. Results and Discussion

The essential oil ratios obtained as a result of five different distillation times (10, 15, 30, 60, and 120 minutes) applied within the scope of the study and the ratios of these data to the total amount of essential oil (yield) are given in Table 1. Research findings show that 80.33% of the total essential oil is obtained in the first 15 minutes of distillation and 100% in 60 minutes. In the 60-120 minute interval,

Table 1. Essential oil content and yield at different distillation times (mean±standard error).

Distillation time (minute)	Essential oil content (%)	Essential oil yield (%)
0-10	1.05±0.00 c	34.43
0-15	2.45±0.00 b	80.33
0-30	2.65±0.00 ab	86.89
0-60	3.05±0.00 a	100.00
0-120	3.05±0.00 a	100.00

*Different letters in the same column indicate a difference of P<0.05 between the means.

Table 2. Essential oil composition at different distillation times (%, mean±standard error).

Essential oil composition	RI[z]	RI[y]	10 min	15 min	30 min	60 min	120 min
α-pinene	1030	1025	0.52±0.01 b	0.53±0.01 ab	0.55±0.02 ab	0.57±0.01 a	0.55±0.00 ab
Sabinene	1122	1110	0.19±0.01	0.19±0.01	0.17±0.03	0.18±0.00	0.18±0.02
δ-3-carene	1132	1122	0.28±0.03	0.23±0.01	0.30±0.03	0.26±0.02	0.28±0.02
Myrcene	1170	1160	2.03±0.02	2.04±0.02	2.05±0.01	2.06±0.00	2.05±0.01
Limonene	1214	1198	96.57±0.05	96.61±0.01	96.52±0.02	96.56±0.02	96.56±0.01
β-phellandrene	1242	1234	0.29±0.00	0.29±0.01	0.29±0.01	0.28±0.01	0.29±0.00
Linalool	1260	1245	0.14±0.02	0.10±0.01	0.12±0.02	0.10±0.01	0.10±0.00

*Different letters at the same line show significant difference at P<0.05.
RI[z]: Calculated from alkane series retention times, RI[y]: Babushok et al. (2011).

Table 3. Essential oil composition and some quality parameters of Navelina peel oil obtained by different extraction methods.

Compounds	HD	CP
α-pinene	0.55±0.01	0.52±0.01
Sabinene	0.16±0.01	0.16±0.01
δ-3-carene	0.32±0.01	0.29±0.02
Myrcene	2.04±0.02	2.04±0.01
Limonene	96.61±0.05	96.53±0.07
β-phellandrene	0.29±0.01	0.29±0.00
Linalool	0.11±0.01	0.09±0.01
Oil content (%)	3.05±0.05 a	0.93±0.02 b
Relative density (20°C)	0.8315±0.00 b	0.8422±0.00 a
Refractive index (20°C)	1.4725±0.00 b	1.4739±0.00 a
Optical activity (20°C)	98.10±0.20 a	96.45±0.25 b

*Different letters at the same line show significant difference at P<0.05.
HD: Hydrodistillation. CP: Cold pressing

which is the last stage of distillation, there was no increase in the total amount of essential oil. The results showed that 60 minutes of Navelina orange essential oil is sufficient for hydrodistillation.

Within the scope of the research, component analysis of essential oils obtained at different distillation times were also performed (Table 2). α-pinene, sabinene, δ-3-carene, myrcene, limonene, and β-phellandrene from monoterpene hydrocarbons and linalool from monoterpene alcohols were detected in orange peel essential oil. Limonene constituted the most important part of the essential oil composition with 96% in all of the samples. The second highest component is myrcene. According to the data obtained, the amount of limonene and myrcene did not change during the times. Monoterpenes (C_{10}) in the orange essential oils have boiling points in the range of 154 to 176°C. It may be due to the fact that the components in the essential oil composition are monoterpene and their chemical structure and properties are close to each other.

In addition, the compositions of essential oils obtained by hydrodistillation (HD) and cold pressing (CP) were evaluated within the scope of the research (Table 3). The data obtained showed that there were significant changes in Navelina orange peel essential oil according to the extraction method. The main components of the peel essential oil obtained by both methods were determined as limonene and myrcene. It was determined that the essential oil component ratios in the Navelina peel essential oil obtained by cold pressing showed similarities with the component ratios of the samples obtained by hydrodistillation, and there were no statistically significant differences between the applications (P>0.05). It was determined that there were significant differences in the amount of oil obtained, density, refractive index, and optical activity values between the essential oil obtained by hydrodistillation and the obtained by cold press (P<0.05). In our study, it was determined that there was no increase in the amount of essential oil at the end of 120 minutes compared to 60 minutes. In this study, in terms of yield according to the hydrodistillation times, it is seen that a 60-minutes period is sufficient to obtain 100% yield to obtain Navelina orange peel essential oil.

In a study conducted on lemon thyme (*Thymus* × *citriodorus*) by Jurevičiūtė et al. (2019), it was shown that hydrodistillation time longer than 60 minutes is useless. In the study by Zhelzajkov et al. (2013), in which the yield and composition of lavender essential oil were examined according to

the distillation time, the maximum oil yield was reached at 60 minutes. Semerdjieva et al. (2019), in their study to determine the essential oil yield of *Hyssopus officinalis* subsp aristatus, obtained a significant amount of essential oil (0.44%) during the first 0-5 minutes of distillation.

In distillation time studies conducted by Jurevičiūtė et al. (2019), Semerdjieva et al. (2019), and Zhelzajkov et al. (2013), it was determined that distillation times could be shorter in terms of yield, similar to our study. It has been observed that hydrodistillation studies, which require a longer time compared to other traditional methods, can be performed in shorter periods. In this way, determining the optimum distillation time specific to the product will also provide an advantage in terms of energy efficiency.

In the study conducted by Ferhat et al. (2016) with HD in 3 varieties of *Citrus sinensis* (Tarocco, Valencia Late, Washington Navel), it was determined that the chemical composition of the essential oil consists mainly of hydrocarbon compounds limonene and β-myrcene. In the essential oil obtained in 180 min HD of three samples, limonene ranged from 92.49% to 95.48%, and β-Myrcene ranged from 1.65% to 1.87%.

In a 3-hour hydrodistillation study with sweet orange (*Citrus sinensis* Osbeck) in a clevenger type device made by Hosni et al. (2010), limonene, β-pinene, and linalool were detected between 96.0-97.3%, 1.45-1.82%, and 0.22-0.04% respectively. It has been reported that the yield and composition differences between sweet orange essential oils can vary depending on genetics, even though they are harvested and processed under the same conditions.

Sawi et al. (2019), in a 4-hour HD study, pointed out that in sweet orange *C. sinensis* (Egypt) dry peel oil, the monoterpene hydrocarbon group, with limonene (86.02%) and myrcene (4.42%) being the main components, is the dominant group in the composition of the essential oil.

According to the results of a study by Manaila et al. (2016), which aimed to compare the properties of essential oil obtained from the fresh peels of five citrus plants by hydrodistillation method, limonene was found in orange (*C. sinensis*) at a rate of 97% and yield of 1.08%. In our study, the main component of Navelina orange peel essential oil was 96% limonene in all of the samples. Similar results have been reported in other studies on orange (Hosni et al., 2010; Dugo and Bonaccorsi, 2014; Ferhat et al., 2016; Gölükcü et al., 2018).

In the 3-hour hydrodistillation study of Hosni et al. (2010), the subspecies of *C. sinensis* Osbeck are Meski (2.31%), Valencia Late (1.89%), and Thomson Navel (1.49%), and Maltaise Blanc (2%) stated that the difference between the yields of dry peel oils of sweet oranges is due to genotype.

Although Ferhat et al. (2016) reported in that the temperature applied in the hydrodistillation method and the long extraction time may change the oil composition, no significant change was detected between the hydrodistillation and cold pressed essential oil compositions in our study. Significant differences were determined in terms of yield in the study carried out by hydrodistillation and cold pressing method on fresh fruit peels *of Citrus sinensis* (L.) Osbeck's Washington Navel, Valencia Late, and Tarocco. It was determined that the yield obtained by hydrodistillation method was higher than that obtained by cold press. In our study, the amount of oil obtained by hydrodistillation method was determined as 3.05% in the fresh peel at 120 minutes, while this rate remained at 0.93% in the cold press.

A measure of essential oil quality is properties such as refractive index, density, and optical activity. Besides the essential oil composition, these values may differ according to the non-volatile components in the chemical composition of the oil. The refractive index, optical activity, and density values of lemon peel oil obtained by hydrodistillation and cold pressing were determined by Ferhat et al. (2007). The results of the study showed that the refractive index, optical activity and density values of the sample obtained by cold pressing were higher than those obtained by hydrodistillation ($P<0.05$). Our findings differed from the literature in terms of optical activity value.

In our study, it was determined that the refractive index and density of the essential oil obtained by hydrodistillation were significantly lower than that obtained by cold pressing ($P<0.05$). The waxes and pigments contained in the orange peel are transferred to the oil during cold pressing. In the peel, which consists of a tissue rich in pigments (chlorophyll and carotenoids), sebaceous glands containing essential oil are unevenly distributed (Giacomo and Raymo, 2014). Ferreira et al. (2020) also reported that oils obtained by cold pressing contain components such as non-volatile carotenoids, and flavonoids. As a matter of fact, while the oil obtained by hydrodistillation was colorless, it had a yellow-orange color obtained by cold pressing. It is thought that these differences may be due to chemical composition differences. As a matter of fact, it was determined by Li et al. (2021) that non-volatile component groups such as coumarin and furanocoumarin were considerably higher in oils obtained by cold pressing compared to those obtained by hydrodistillation. In addition, these values could be changed by any adulteration. Dilution of the most valuable citrus oils with inexpensive sweet orange terpenes results in increased optical rotation (Bonaccorsi et al., 2014).

It was determined that there were significant differences in the amount of oil obtained, density, refractive index, and optical activity values between the essential oil obtained by hydrodistillation and the obtained by cold press ($P<0.05$). This difference shows that depending on the extraction method to be applied for sweet orange peel oil with different properties can be produced.

5. Conclusion

Essential oil compositions in raw plant materials can be significantly affected by factors such as the age of the plant, the used part, the harvest time, the type of harvest, and the drying method, especially the species and variety. Standardized limonene is an important issue in the essential oil industry. Limonene can be obtained from the peels of sweet oranges, which contain as high as 96% limonene. The standardized product can be used in many areas.

Within the scope of the research, the effect of distillation time on orange essential oil composition was investigated. Findings revealed that when distillation time is taken into account, a product with a different composition in terms of limonene was not obtained. It has been determined that there are significant differences in parameters such as density, refractive indices, optical activity, and color of the oils obtained by hydrodistillation and cold pressing, and it has been determined that different extraction methods can be selected according to the purpose of use.

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References

Anastasopoulou, E, Graikou, K, Ganos, C, Calapai, G. & Chinou, I. (2020). Pimpinella anisum seeds essential oil from Lesvos Island: Effect of hydrodistillation time, comparison of its aromatic profile with other samples of the Greek market. Safe use. *Food and Chemical Toxicology*, 135(2020):110875.

Angiolillo L., Nobile, M.A.D. & Conte, A. (2015). The extraction of bioactive compounds from food residues using microwaves. *Current Opinion in Food Science*, 5:93-98.

Aygören, E. (2021). *Ürün Raporu Turunçgiller 2021*. Tarimsal Ekonomi ve Politika Geliştirme Enstitüsü TEPGE. Tepge Yayın No: 333. 2021, ISBN: 978-605-7599-72-8 (in Turkish).

Babushok, V.I., Linstrom, P.J., & Zenkevich, I.G. (2011). Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data*, 40(4):043101.

Bonaccorsi, I., Schipilliti, L. & Dugo, G. (2014). Adulteration of Bergamot Oil. *Citrus bergamia bergamot and its derrivatives*. Dugo G, Bonaccorsi I (eds). CRC Press: New York, 2014; Volume 51, pp. xvii.

Dugo, G. & Bonaccorsi, I. (2014). *Citrus bergamia bergamot and its derrivatives*. Dugo G, Bonaccorsi I (eds). CRC Press: New York, Volume 51, pp. xvii.

Durazzini, A.M.S., Machado, C.H.M., Fernandes, C.C. & Miranda, M.L.D. (2019). Chemical composition and effect of hydrodistillation times on the yield of essential oil from *Eugenia pyriformis* leaves. The *Electronic Journal of Chemistry*,11(5):334-338.

Erasto, P. & Viljoen, A.M. (2008). Limonene - A Review: Biosynthetic, ecological and pharmacological relevance. *Natural Product Communications*, 3(7):1193-1202.

Ferhat, M.A., Meklati, B.Y. & Chemat, F. (2007). Comparison of different isolation methods of essential oil from citrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. *Flavour and Fragrance Journal*, 22:494–504.

Ferhat, M.A., Boukhatem, M.N., Hazzit M., Meklati, B.Y. & Chemat, F. (2016). Cold pressing, hydrodistillation and microwave dry distillation of citrus essential oil from Algeria: A Comparative Study. *Electronic Journal of Biology*, S1: 30-41.

Ferreira, G., Sobral, L., Barreto, D.W. & Calado, V. (2020). Thermal and compositional analysis of orange essential oil obtained from citrus ındustry waste. *Journal of Advanced Thermal Science Research*, 7:48-55.

Giacomo, A.D. & Raymo, V. (2014). Transformation Industry. *Citrus bergamia bergamot and its derrivatives*. Dugo G, Bonaccorsi I (eds). CRC Press: New York, 2014; Volume 51, pp. xvii.

Gölükcü, M., Toker, R., Tokgöz, H. & Turgut Yıldız, D. (2015). Bitter orange (*Citrus aurantium* L.) peel essential oil compositions obtained with different methods. *Derim*, 32(2):161- 170.

Gölükcü, M., Tokgöz, H. & Turgut Yıldız, D. (2018). Effect of distillation time on essential oil compositions of bay leaf (*Laurus nobilis* L.). *Food and Health*, 4(1):37-42.

Hilali, S., Tixier, A.S.F., Ruiz, K., Hejjaj, A., Nouh, F.A., Idlimam, A., Bily, A., Mandi, L. & Chemat, F. (2019). Green extraction of essential oils, polyphenols, and pectins from orange peel employing solar energy: toward a zero-waste biorefinery. *ACS Sustainable Chemistry & Engineering*, 7:11815-11822.

Hosni, K. Zahed, N., Chrif, R., Abid, I., Medfei, W., Kallel, M., Brahim, N.B. & Sebei, H. (2010). Composition of peel essential oils from four selected Tunisian citrus species: Evidence fort he genotypic influence. *Food Chemistry*, 123(2010): 1098-1104.

Jurevičiūtė, R., Ložienė, K., Bruno, M., Maggio, A. & Rosselli, S. (2019). Composition of essential oil of lemon thyme (*Thymus* × *citriodorus*) at different hydrodistillation times. *Natural Product Res*earch, 33(1):80-88.

Karr, L.L. & Coats, J.R. (1988). Insecticidal properties of d-Limonene. *Journal of Pesticide Science*, 13:287-290.

Kırbaşlar, F.G., Kırbaşlar S.I. & Dramur U. (2001). The compositions of Turkish bergamot oils produced by cold-pressing and steam distillation. *Journal of Essential Oil Research*,13(6):411-415.

Li, G., Xiang, S., Pan, Y., Long, X., Cheng, Y., Han, L. & Zhao, X. (2021). Effects of cold-pressing and hydrodistillation on the active non-volatile components in lemon essential oil and the effects of the resulting oils on aging-related oxidative stress in mice. *Frontiers in Nutrition*, 8:689094.

Manaila E., Berechet M.D., Stelescu M.D., Cracuin G., Mihaiescu D.E., Purcareanu B., Calinescu I., Fudulu A., Radu M. (2016). Comparation between chemical compositions of some essential oils obtained by hydrodistillation from citrus peels. *Revista de Chimie*, 67(1):107-112.

Maurya, A., Prasad, J., Das, S. & Dwivedy, A. K. (2021). Essential oils and their application in food safety. *Frontiers in Sustainable Food Systems*, 5:653420.

Ngan, T.T.K., Nguyen, O.B., Muoi, N.V., Truc, T.T. & My V.T.N. (2020). Chemical composition and antibacterial activity of orange (*Citrus sinensis*) essential oils obtained by hydrodistillation and solvent

free microwave extraction. *Materials Science and Engineering*, 991(2020):012023.

Özek, G., Demirci, F., Özek, T., Tabanca, N., Wedge, D.E., Khan, S.I., Başer, K.H.C., Duran, A. & Hamzaoglu, E. (2010). Gas chromatographic–mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity. *Journal of Chromatography*, A, 1217:741-748.

Sawi, S.A.E., Ibrahim, M.E., El-Rokiek, K.G. & El-Din S.A.S. (2019). Allelopathic potential of essential oils isolated from peels of three citrus species. *Annals of Agricultural Sciences,* 64(2019):89–94.

Semerdjieva, I.B., Burducea, M., Astatkie, T., Zheljazkov, V.D. & Dincheva, I. (2019). Essential oil composition of Rutagraveolens L. fruits and *Hyssopus officinalis* subsp. aristatus (Godr.) Nyman biomass as a function of hydrodistillation time. *Molecules*, 24:4047.

Shan, Y. (2016). Functional Components of Citrus Peel. In Comprehensive Utilization of Citrus By-Products*,* pp. 1-13, ISBN 978-0-12-809785-4.

TS (2009). ISO 280: Eteri yağlar- Kırılma indisi tayini (in Turkish).

TS (2012a). ISO 279: Eteri yağların yoğunluk venisbi yoğunluklarının tayini (in Turkish).

TS (2012b). ISO 592: Eteri yağlar- Optik çevirme açısı tayini-Polarimetrik metot (in Turkish).

TSE (Turkish Standardization Institute). (2011). TSE EN ISO 6571-Baharatlar, çeşniler ve tıbbi bitkiler - uçucu yağ muhtevasının tayini (hidrodistilasyon yöntemi). 2011, Türk Standartları Enstitüsü; Ankara, Türkiye (in Turkish).

Vieira, A.J., Beserra, F.P., Souza, M.C., Totti, B.M. & Rozza, A.I. (2018). Limonene: Aroma of innovation in health and disease. *Chemico-Biological Interactions*, 283(2018):97-106.

Zheljazkov, V.D., Cantrell, C.L., Astatkie, T. & Jeliazkova, E. (2013). Distillaton time effects on lavender essential oil yield and composition. *Journal of Oleo Science*, 62:195-199.

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RESEARCH PAPER

HORTICULTURAL STUDIES

The Effects of Liquid Biogas Digestate on Yield and Mineral Nutrition of Cucumber Growing in Greenhouse

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Abstract

This study aimed to investigate the effects of a liquid fraction of digestate obtained from different biogas plants on the growth and mineral nutrition of cucumber plants under greenhouse conditions. For this purpose, Liquid Biogas Digestates (LBD) obtained from two different plants (A-B) with different properties were applied to pots with 10 kg of soil in 5 different doses (0, 20, 40, 60, and 80 t ha^{-1}) and the effects of the treatments were observed. As a result of the research, the highest yields increased 24.6% for digestate A in A5 (80 t ha^{-1}) and 29% for digestate B in B3 (40 t ha^{-1}) compared to control. While LBD contributed to the increase of N, Ca, Zn, Cu, and Mn concentrations in the leaf samples, it was observed that the dose increase did not have a linear effect on N, Ca, Zn, Cu, and Mn concentrations in the leaf samples. It is thought that liquid biogas wastes produced in biogas plants have positive effects on fruit yield, agricultural practices can be taken as the basis for the disposal of these wastes and the use of liquid biogas residues in soils by eliminating potential risks can provide significant benefits.

1. Introduction

Biogas is a colorless, odourless, lighter-than-air and flammable gas mixture that is the end product of the decomposition of organic waste and residues by various bacteria in an anaerobic environment (Weiland, 2010). Its composition includes 50-75% methane, 25-50% carbon dioxide, hydrogen sulphide (H_2S), hydrogen (H_2), ammonia (NH_3) (1-2%) and trace other gases as oxygen and nitrogen (Atelge et al., 2020). The waste that is released after biogas generation can be converted into a significantly more valuable fertilizer that is required for organic farming, so preventing pollution of the environment. Environmental health benefits from this conversion, especially in rural regions (Chang et al., 2011). The amount of solid and liquid digestate produced daily by an average-sized plant during the biogas production phase is approximately 1500 m^3 and approximately 95% of this waste is liquid fraction of digestate. It becomes quite difficult to dispose of such a large amount of liquid digestate. As the amount of these liquid biogas digestates, which are usually stored in lagoons or pools, increases, problems such as flies and bad odours cause environmental pollution. These wastes can seep through the soil and mix with groundwater and contaminating groundwater resources. For these reasons, the accumulation of these wastes cannot be continued for a long time. Although the composition of liquid biogas digestates is generally 93-99% water and 1-7% dry matter (Lukehurst et al., 2010), it contains significant amounts of mineral elements, enzymes and amino acids, as well as significant amounts of organic matter (OM) with a relatively low C/N ratio (Alburquerque et al., 2012).

There are some studies on the use of liquid biogas digestate and sludge in agriculture. The application of biogas sludge increases the amount of N-P-K in the soil, has a positive effect on parameters such as protein, soluble sugars and β-

carotene in the plant, and also plays an important role in increasing the number of culturable bacteria and actinomycetes in the soil (Yu et al., 2010). Application of biogas sludge to plants increased plant height, root length, chlorophyll content, stomatal conductance and water use (Xu et al., 2013). Studies have shown that liquid biogas digestate and sludge application increased to plant growth, yield and nutrient uptake (Abubaker et al., 2012; Ferdous et al., 2018; İbil, 2019; Yaylacı and Erdal, 2021; Adamovics and Sivicka, 2023). Liquid biogas digestate and sludge had positive effects on the number of spikes per square meter, thousand grain weight, number of grains per spike, flag leaf area and grain yield, hectolitre weight, ash and protein content in cereals (Yaraşır et al., 2018; Karaman and Türkay, 2022).

This study was conducted to determine cucumber plant height, fresh and dry weight, fruit length, fruit diameter, yield and mineral nutrition status of the digestate fraction obtained from different biogas plants. Thus, it was aimed to reveal the possibilities of using problematic waste in the agricultural sector.

2. Material and Method

This research was carried out in the greenhouses of Akdeniz University Faculty of Agriculture in 2022. In the experiment, which was conducted under the pot conditions in the greenhouse, 5 different doses (0, 20, 40, 60 and 80 t ha^{-1}) of Liquid Biogas Digestate (LBD) obtained from two different biogas plants (A and B) were applied to the soils. The effects of the treatment doses of liquid biogas digestate on growth, yield and mineral nutrition were attempted to be determined (Table 1). LBD-A is a biogas plant located in Aksaray and produces biogas with a capacity of 1.067 MW using all agricultural waste and animal manure in the immediate vicinity of the plant. LBD-B is a biogas plant located in Malatya and produces biogas with a capacity of 3.12 MW using poultry manure, broiler manure and solid manure.

Greenhouse cultivation was carried out between September 15 and December 13, 2022, and the temperature change in the greenhouse during the cultivation period was recorded (Figure 1). It was observed that the day-night temperature differences increased due to the autumn growing season, but this situation did not reach levels that would prevent production.

Some soil properties in experiment were as follows: pH: 6.99, $CaCO_3$: 27.12%, loamy loam in texture, EC: 0.31 dS m^{-1}, organic matter content: 0.87%, total N: 0.30%, available P: 38.23 mg kg^{-1}, exchangeable K: 0.18 meq 100 g^{-1}, exchangeable Ca: 33.18 meq 100 g^{-1}, exchangeable Mg: 7.06 meq 100g^{-1}, available Zn: 0.38 mg kg^{-1}, available Mn: 4.53 mg kg^{-1} and available Cu: 1.48 mg kg^{-1}.

Table 1. Treatment rates of Liquid Biogas Digestates (LBD) from different biogas plants.

Treatment	Dose	Treatment	Dose
A1	0 t ha^{-1} LBD	B1	0 t ha^{-1} LBD
A2	20 t ha^{-1} LBD	B2	20 t ha^{-1} LBD
A3	40 t ha^{-1} LBD	B3	40 t ha^{-1} LBD
A4	60 t ha^{-1} LBD	B4	60 t ha^{-1} LBD
A5	80 t ha^{-1} LBD	B5	80 t ha^{-1} LBD

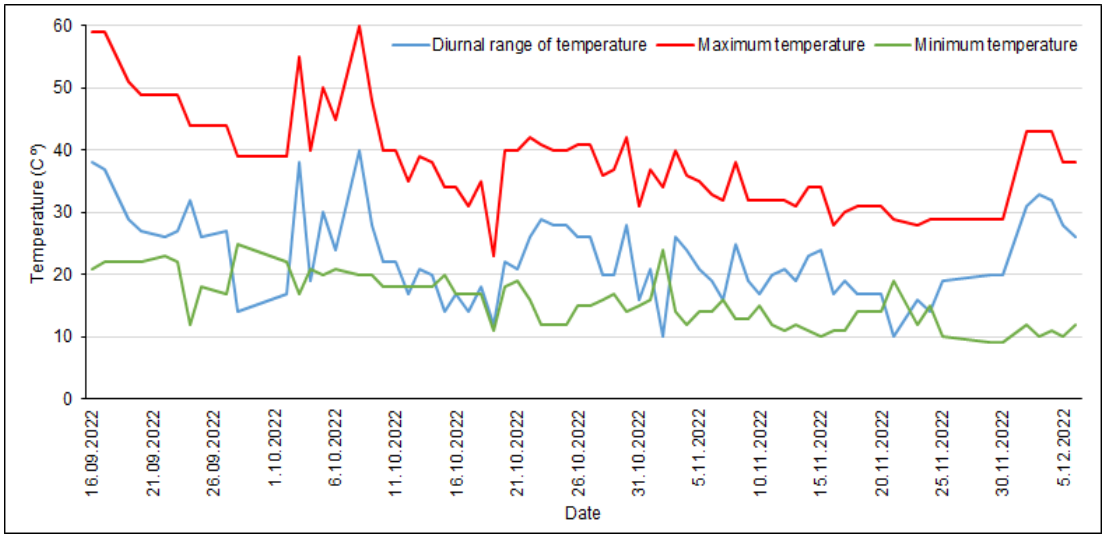

Figure 1. Temperature change in the greenhouse during the growing period.

The test plant used in the study was the cucumber variety Silor type YT-195. Silor type cucumber (YT-195) has short internodes, productive, dark green, long stem, high quality, 10-12 cm heigh, very good taste and flavour, long shelf-life. The experiment was designed according to the randomized block design with 4 replications, seedlings were planted separately for 2 different liquid biogas digestates in pots with 10 kg soil, with 1 plant in each pot, and all cultural procedures (irrigation, spraying, plant protection etc.) were applied equally to all pots during the cultivation process. During the growing period, 160 kg ha^{-1} N, 140 kg ha^{-1} P_2O_5, 290 kg ha^{-1} K_2O were applied equally to all pots 1-2 times a week with irrigation. Other plant nutrients were not applied to observe the effect of nutrient content of liquid biogas digestate. The application of liquid biogas digestates to the soil started 1 week after the seedlings were transplanted to the soil and was applied a total of 4 times, once every 14 days. In the control treatment, only water was applied. Some analytical results of the liquid biogas digestate used as treatment material are given in Table 2.

Plant height, plant fresh and dry weight, fruit length (cm), fruit diameter (mm) and fruit yield (g $plant^{-1}$) were determined in cucumber plants. Cucumber fruit length was measured using a ruler from one end to another, while fruit diameter was measured in three different locations on the fruit with caliper. Fruit weight was measured by placing it on an electronic scale and recording the weight.

In the experiment, leaf and fruit samples of cucumber plants were collected and after the necessary physical measurements, taken to the laboratory, washed, dried at 65°C to constant weight, then ground and prepared for analysis (Kacar and İnal, 2008). For plant analyses, total Nitrogen (N) was determined in cucumber leaf samples using the modified Kjeldahl Method (Bremner, 1965). In addition, for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn) and copper (Cu) analyses, plant samples were wet combusted and read by ICP-OES (Perkin Elmer-Optima 7000 DV) as reported by Soltanpour and Workman (1981).

Soil samples were analysed for pH (Jackson, 1967), lime ($CaCO_3$) (Evliya, 1964), electrical conductivity (Anonymous, 1988), composition (Bouyoucos, 1955), organic matter (Black, 1965), total N (Black, 1957), available P (Olsen and Sommers, 1982), extractable K, Ca and Mg, available Zn, Cu and Mn (Lindsay and Norvell, 1978; Knudsen et al., 1982).

In liquid biogas digestates, pH and EC were determined by pH-meter and EC-meter, and organic matter was determined by dry combustion at 550°C (Kacar, 1972), the dry matter content was measured by keeping it at 70°C until reaching stable weight, total nitrogen by devarda (Liao, 1981), P, K, Ca, Mg, Zn, Mn and Cu were determined by treating 1 g of fertilizer sample with 50 ml of water and 1 ml of concentrated nitric acid, adding to 100 ml, filtering and reading in an ICP-OES (Inductively Coupled Plasma-Atomic Emission Spectrometry-Perkin Elmer 700DV).

The results obtained from the laboratory analyses and measurements were subjected to Duncan's multiple comparison test by analysis of variance in the SPSS 17.0 package program (Yurtsever, 1984).

3. Results and Discussion

Some measurements and analyses were carried out on the leaf and fruit samples of the cucumber plant. The effects of two different liquid biogas digestates on plant height and plant fresh weight of cucumber plants were found to be statistically significant (Table 3), while plant dry weight values were not affected by the treatments.

LBD-A treatment A4 (60 t ha^{-1}) together with control produced the highest plant height, while the A5 (80 t ha^{-1}) produced the highest fresh weight. In the LBD-B treatment, the highest values for plant height and fresh weight were obtained with B3 (40 t ha^{-1}). There was no linear relationship between increasing doses and changing fresh and dry weights. LBD-A and LBD-B materials have different properties and show different changes in fresh and dry weight values. Plant height is

Table 2. Some analyses properties of Liquid Biogas Digestates (LBD) used in the experiment.

Parameter	LBD-A	LBD-B
pH	9.17	8.58
EC (dS m^{-1})	22.2	14.53
Organic matter (%)	1.46	3.7
Dry matter (%)	5.51	2.08
N (%)	1.47	2.48
P (%)	0.022	0.019
K (mg kg^{-1})	446.94	338.91
Ca (mg kg^{-1})	97.20	60.20
Mg (mg kg^{-1})	23.16	5.76
Zn (mg kg^{-1})	2.63	2.96
Mn (mg kg^{-1})	3.88	1.63
Cu (mg kg^{-1})	0.80	2.20

EC:Electrical conductivity, N:Nitrogen, P:Phosphorus, K:Potassium, Ca:Calcium, Mg:Magnesium, Zn:Zinc, Mn:Manganese, Cu:Copper

Table 3. Effects of different Liquid Biogas Digestates (LBD) on plant height, fresh and dry weight of cucumber plants.

Treatment	LBD-A			LBD-B		
	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Fresh weight (g)	Dry weight (g)
1	151.5±8.06^{a}	229.3±7.93^{b}	9.3±1.66	147.8±2.84^{b}	198.6±6.33^{c}	7.87±0.87
2	122.3±4.03^{b}	167.0±6.34^{c}	6.1±1.10	156.5±7.67^{ab}	241.8±5.87^{b}	7.85±1.95
3	129.3±3.07^{b}	233.9±4.66^{b}	7.7±1.03	170.3±4.77^{a}	279.8±5.24^{a}	7.61±2.47
4	144.3±4.09^{a}	163.9±7.38^{c}	5.9±0.58	151.0±3.81^{b}	150.5±7.06^{d}	5.83±1.22
5	123.3±2.02^{b}	271.0±7.81^{a}	8.6±2.38	145.5±4.56^{b}	214.4±4.90^{c}	8.45±0.66
F value	7.72***	44.69***	1.03^{ns}	3.91*	66.28***	0.39^{ns}

*p<0.05, ***p<0.001, ns: not significant

Table 4. Effects of different Liquid Biogas Digestates (LBD) on macro element concentrations in in cucumber leaf samples.

Treatment	LBD-A (%)				
	N	P	K	Ca	Mg
A1	2.55±0.15^{ab}	0.30±0.01^{a}	2.58±0.05^{a}	2.26±0.02^{c}	1.18±0.06
A2	2.32±0.73^{b}	0.24±0.01^{b}	2.23±0.07^{b}	2.47±0.17^{ac}	1.17±0.11
A3	2.66±0.04^{a}	0.24±0.01^{b}	2.12±0.02^{b}	2.29±0.09^{bc}	1.16±0.18
A4	2.67±0.09^{a}	0.24±0.02^{b}	2.26±0.06^{b}	2.60±0.01^{ab}	1.01±0.01
A5	2.85±0.09^{a}	0.25±0.01^{b}	2.22±0.09^{b}	2.75±0.10^{a}	0.99±0.08
F value	4.13**	7.68***	8.81***	4.21**	0.80^{ns}
Treatment	LBD-B (%)				
	N	P	K	Ca	Mg
B1	4.09±0.11^{a}	0.22±0.01	2.65±0.19	2.48±0.16	0.95±0.13
B2	4.16±0.21^{a}	0.21±0.01	2.47±0.08	2.56±0.21	1.10±0.15
B3	4.60±0.13^{a}	0.26±0.03	2.51±0.13	3.12±0.19	0.92±0.11
B4	2.80±0.26^{b}	0.27±0.03	2.61±0.19	3.10±0.37	1.03±0.13
B5	2.71±0.11^{b}	0.21±0.01	2.54±0.08	2.64±0.25	0.91±0.11
F value	24.90**	2.10^{ns}	0.25^{ns}	1.54^{ns}	0.40^{ns}

**p<0.01, ***p<0.001, ns: not significant. N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium.

Table 5. Effects of different Liquid Biogas Digestates (LBD) on microelement concentrations in cucumber leaf samples.

Treatment	LBD-A (mg kg^{-1})			LBD-B (mg kg^{-1})		
	Zn	Mn	Cu	Zn	Mn	Cu
1	6.72±0.65^{a}	33.27±3.41	2.14±0.32^{b}	5.49±0.28^{b}	22.62±1.92	2.73±0.27
2	6.26±0.32^{a}	29.31±1.31	4.61±0.69^{a}	8.17±0.82^{a}	26.24±3.25	2.33±0.15
3	4.67±0.40^{b}	29.42±3.12	1.73±0.32^{b}	8.45±0.70^{a}	27.55±2.55	1.95±0.29
4	4.89±0.39^{b}	25.73±1.34	2.42±0.18^{b}	8.34±0.56^{a}	28.54±4.06	2.24±0.47
5	4.70±0.18^{b}	27.99±6.48	1.85±0.30^{b}	5.62±0.38^{b}	25.83±0.55	1.66±0.22
F value	5.37***	0.56^{ns}	8.78***	6.87**	0.67^{ns}	1.79^{ns}

**p<0.01, ***p<0.001, ns: not significant. Zn: Zinc, Mn: Manganese, Cu: Copper.

increased by biogas liquid digestate and sludge, according to Ferdous et al. (2020) and Ronga et al. (2019). Baştabak (2019) found that biogas-fermented waste increased root and stem length in lettuce. Abubaker et al. (2012) reported that biogas sludge contributed to the increase in fresh weight of plants. Kouřimská et al. (2012) reported that the use of biogas liquid digestate improved the quality and yield of vegetables.

The effects of different liquid biogas digestates on macro element concentrations (%) in cucumber plants are shown in Table 4. Regarding the effect of LBD-A treatments on macro-element concentrations in cucumber leaf samples, the effect of treatments on all elements except Mg was found to be statistically significant. The highest values for N in cucumber leaf samples were obtained from A3, A4 and A5 treatments, while the control treatments gave the maximum value for P and K contents. The liquid biogas digestate had no effect on the P and K contents of the leaves. It was observed that the effect of LBD-B treatments on macroelement contents of cucumber leaf samples was only on N concentration and B1, B2 and B3 treatments gave maximum values. LBD-A material was more effective than LBD-B material for N and Ca in cucumber leaf samples. Biogas sludge is a readily available source of ammonium nitrogen in soil, which can increase plant N concentration and contribute to growth (Moller et al., 2008; Yu et al., 2010; Nkoa, 2014; Koszel and Lorencowicz, 2015). Applying biogas sludge to the soil improves its organic matter content, nutrient concentrations, water-holding capacity, and nitrogen use efficiency (Akanbi et al., 2010).

The effects of different liquid biogas digestates on micro-element concentrations (mg kg^{-1}) in cucumber leaf samples are shown in Table 5. The effects of LBD-A treatments on Zn and Cu in microelement concentrations in cucumber leaf samples were found to be statistically significant. In cucumber leaf samples, the highest values for Zn

Table 6. Effects of different Liquid Biogas Digestates (LBD) on fruit length, fruit diameter, yield per plant in cucumber.

Treatment	LBD-A			LBD-B		
	Fruit height (cm)	Fruit diameter (mm)	Yield (g plant^{-1})	Fruit height (cm)	Fruit diameter (mm)	Yield (g plant^{-1})
1	11.7±0.25	28.8±0.95	210.1±2.98^{c}	12.4±0.66	38.1±1.46^{a}	198.6±4.94^{cd}
2	12.9±0.42	29.2±0.52	194.2±2.99^{c}	12.1±0.16	29.1±0.76^{c}	245.4±6.87^{b}
3	12.2±0.33	30.6±1.62	254.2±7.39^{b}	13.1±0.39	34.0±2.15^{ab}	279.8±5.24^{a}
4	13.5±0.56	33.3±1.65	193.8±4.18^{c}	11.8±0.48	36.7±0.85^{ab}	195.5±5.50^{d}
5	13.0±0.57	33.1±2.29	278.5±8.90^{a}	11.8±0.36	33.5±0.91^{b}	214.4±6.69^{c}
F value	2.68^{ns}	1.88^{ns}	43.29***	1.39^{ns}	6.77**	36.52***

**p<0.01, ***p<0.001, ns: not significant.

Table 7. Effects of different Liquid Biogas Digestates (LBD) on macro element concentrations in cucumber fruits.

Treatment	LBD-A (%)				
	N	P	K	Ca	Mg
A1	1.56±0.09^{ab}	0.43±0.02^{b}	2.79±0.07^{b}	0.61±0.07^{bc}	0.32±0.02^{b}
A2	1.63±0.03^{a}	0.55±0.06^{a}	3.68±0.22^{a}	0.71±0.01^{ab}	0.41±0.01^{a}
A3	1.41±0.04^{b}	0.42±0.01^{b}	2.86±0.05^{b}	0.54±0.03^{c}	0.32±0.01^{b}
A4	1.42±0.03^{b}	0.48±0.02^{ab}	3.49±0.29^{a}	0.78±0.03^{a}	0.34±0.02^{b}
A5	1.54±0.05^{ab}	0.55±0.03^{a}	3.61±0.15^{a}	0.68±0.02^{ab}	0.35±0.01^{b}
F value	3.56*	3.40*	5.67**	6.04**	6.69**
Treatment	LBD-B (%)				
	N	P	K	Ca	Mg
B1	1.53±0.03^{b}	0.41±0.02^{b}	3.40±0.13	0.59±0.03	0.31±0.03
B2	1.31±0.02^{d}	0.45±0.03^{b}	3.41±0.20	0.61±0.03	0.33±0.03
B3	1.69±0.02^{a}	0.56±0.03^{a}	3.83±0.20	0.59±0.01	0.37±0.03
B4	1.42±0.04^{c}	0.49±0.02^{a}	3.43±0.15	0.55±0.04	0.33±0.02
B5	1.30±0.03^{d}	0.46±0.02^{b}	3.31±0.08	0.60±0.01	0.36±0.02
F value	30.76***	5.04**	1.62^{ns}	0.87^{ns}	0.97^{ns}

**p<0.01, ***p<0.001, ns: not significant. N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium.

were obtained from the A1 and A2 treatments, while the highest values for Mn were obtained from the A2 treatment. The effect of treatments on Mn content was not found to be significant. The effect of LBD-B treatments on the microelement concentrations of cucumber fruit was found to be substantial with respect to Zn content, with the B2, B3, and B4 treatments providing the highest values of Zn content. Liquid biogas digestates are rich in plant nutrients and organic contents that can be used in agricultural production (Bauer et al., 2009), and can increase macro and microelement contents in soils and plants (Chiew et al., 2015).

The effects of different liquid biogas digestates on cucumber fruit length, fruit diameter and yield per plant are shown in Table 6. Although the LBD-A treatments had no effect on cucumber fruit length and fruit diameter, the yield per plant values were found to be statistically significant. The highest yield values were produced from A5 (80 t ha^{-1}), which was the maximum dose and A5 increased yield by 24.6% compared to the control (A1). Fruit diameter and yield values in the LBD-B treatment were found to be statistically significant; in contrast, the control treatment had the highest fruit diameter value. B3 (40 t ha^{-1}) had the highest yield value per plant, and it increased yield by 29% when compared to treatment B1. The effect of LBD-B material is more effective than LBD-A material on fruit characteristics. Biogas digestates had a significant effect on cucumber yield (Adamovics and Sivicka, 2023). Yaraşır et al. (2018) and Rózyło et al. (2017) reported that liquid biogas digestates had a significant effect on wheat yield. Makadi et al. (2008) reported that the use of liquid biogas digestates increased the yield, protein and amino acid content of soybean. Li et al. (2023) reported that biogas sludge applied at 3% (V/W) had the best ability to promote growth and suppress disease without the risk of soil salinization.

The effects of the different liquid biogas digestates on the concentrations of the macro-elements (%) in the cucumber fruit are shown in Table 7. The effect of LBD-A treatments on macro-element concentrations in cucumber fruit was found to be statistically significant. A2 had the highest concentrations of N, P, and Mg in the cucumber leaf samples; A2, A4, and A5 had the highest concentrations of K, and A4 had the highest concentrations of Ca. LBD-B treatments were effective only on N and P concentrations in cucumber macroelement, B3 was effective on N concentration and B3 and B4 were effective on P concentration. Fruit's nutrient content was enhanced by the of liquid biogas digestates, which provided enough nutrients for the plants. The use of liquid biogas digestates increases plant biomass, magnesium content and photosynthetic efficiency (Xu et al., 2013). Liquid biogas digestates contribute to plant N recovery, and this contribution is higher for liquid waste than for biogas slurry (Lukehurst et al., 2010). This nitrogen-rich material contributes to plant growth and nitrogen balance. Liquid biogas digestates can have similar effects to chemical

Table 8. Effects of different liquid biogas digestates on micro element concentrations in cucumber fruit.

Treatment	LBD-A (mg kg^{-1})			LBD-B (mg kg^{-1})		
	Zn	Mn	Cu	Zn	Mn	Cu
1	7.08±0.14^{d}	3.32±0.39^{b}	2.11±0.12^{c}	14.83±0.42^{c}	7.95±0.33^{b}	11.78±0.66
2	11.84±0.51^{c}	5.02±0.49^{b}	3.00±0.35^{c}	16.41±0.47^{c}	8.04±0.50^{b}	11.76±0.77
3	12.77±1.20^{c}	7.81±0.29^{a}	8.49±0.69^{b}	29.06±0.86^{a}	10.02±0.13^{a}	13.80±0.72
4	16.60±0.38^{b}	8.73±0.44^{a}	12.29±0.21^{a}	19.70±0.84^{b}	8.47±0.41^{b}	12.96±0.60
5	23.92±1.14^{a}	8.80±1.11^{a}	12.80±0.44^{a}	16.71±0.84^{c}	8.65±0.27^{b}	12.59±0.76
F value	62.84***	15.80***	148.39***	63.76***	5.69**	1.49^{ns}

**p<0.01, ***p<0.001, ns: not significant. Zn: Zinc, Mn: Manganese, Cu: Copper.

fertilizers on shoot and root dry mass and nutrient content (C, N, and P) (Barbosa et al., 2014). Biogas digestates provided high levels of P, K, and Mg in kohlrabi (Losak et al., 2014). Liquid biogas digestates provided the highest levels of macronutrients and unsaturated fatty acids in winter oilseed rape (Koszel et al., 2020).

The effects of different liquid biogas digestates on the concentrations of micro-elements (mg kg^{-1}) in cucumber fruits are shown in Table 8. The effect of the LBD-A treatments on the concentrations of microelements in the cucumber fruit samples was found to be statistically significant. The highest value for Zn content in cucumber fruit samples was obtained from A5, while the highest values for Mn content were obtained from A3, A4 and A5. A4 and A5 had the highest values for Cu content of fruits. It has been determined that the effect of LBD-B applications on the microelement contents of cucumber fruits was significant, and that B3 had the maximum values for Zn and Mn contents in the fruits. LBD treatments provided microelement enrichment in the fruit due to the microelement concentrations in their compositions. Liquid biogas digestate contains sufficient levels of plant nutrients (Yadav and Garg, 2016). Başţabak and Koçar (2020) stated that the contents of liquid biogas digestates are rich in nutrients useful for plants. Yaraşır et al. (2018) reported that liquid biogas digestates can be used as an alternative fertilizer for soil fertility and plant growth.

4. Conclusion

Liquid biogas digestates, which are anaerobic fermentation products of organic wastes used in biogas production facilities, are usually accumulated in large lagoons and ponds and these materials carry some environmental risks (odour, disease transmission by vectors, leakage into groundwater, etc.). Researches conducted in this context show that liquid biogas digestates can be used in agricultural areas by taking the necessary precautions. In this study, it was determined that treatment doses of 40-80 t ha^{-1} were effective on plant growth (plant height, fresh and dry weight, fruit length, fruit diameter), yield and some mineral nutrient values of fermented liquid digestates obtained from different biogas plants and dose increases did not have a linear effect on the studied parameters. It was observed that liquid biogas digestates do not have a homogeneous structure and may have different properties due to the fact that the materials produced in each biogas plant consist of materials with different contents. In order to prevent the environmental risks of liquid biogas digestates, alternative methods of management and treatments that contribute to production should be planned in advance. Pasteurization can be done against possible pathogen risks and can be used with dilution to reduce the risk of salinity. Product-based research should be carried out on the use of these products in agriculture and possible risks should be determined.

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References

Abubaker, J., Risberg, K., & Pell, M. (2012). Biogas residues as fertilisers–Effects on wheat growth and soil microbial activities. *Applied Energy*, 99:126-134.

Adamovics, A., & Sivicka, I. (2023). Influence of biogas digestate, wood ash and their mixtures on the yield and quality of cucumbers. *AGROFOR International Journal*, 8(1):68-75.

Akanbi, W.B., Togun, A.O., Adediran, J.A., & Lupeju, E.A.O. (2010). Growth, dry matter and fruit yield components of okra under organic and inorganic sources of nutrients. *American-Eurasian Journal of Sustainable Agriculture,* 4:1-13.

Anonymous, (1988). Fruit, vegetables and products - nitrite and nitrate definition - molecular absorption spectrophotometric method. Turkish Standard, ICS 67.080, TS6183.

Alburquerque, J.A., De LaFuente, C., Campoy, M.M., Carrascoa, L., Nájerab, I., Baixaulib, C., Caravacaa, F., Roldána, A., Cegarraa, J., & Bernal, M.P. (2012). Agricultural use of digestate for horticultural crop production and improvement of soil properties. *European Journal of Agronomy*, 43:119-128.

Atelge, M.R., Krisa, D., Kumar, G., Eskicioglu, Ç., Nguyen, D.D., Chang, S.W., Atabani, A.E., Al-Muhtaseb, A.H., & Unalan, S. (2020). Biogas production from organic waste: recent progress and perspectives. *Waste and Biomass Valorization.* 11:1019–1040.

Barbosa, D.B.P., Nabel, M., & Jablonowski, N.D. (2014). Biogas-digestate as nutrient source for biomass production of Sida hermaphrodita, Zea mays L. and Medicago sativa L. *Energy Procedia*, 59:120-126.

Başţabak, B. (2019). Investigation of the effect of fermented fertilizer from biogas plant in lettuce cultivation. MSc Thesis, Ege University, İzmir.

Baştabak, B., & Koçar, G. (2020). A review of the biogas digestate in agricultural framework. *Journal of Material Cycles and Waste Management*, 22:1318-1327.

Bauer, A., Mayr, H., Hopfner-Sixt, K., & Amon, T. (2009). Detailed monitoring of two biogas plants and mechanical solid-liquid separation of fermentation residues. *Journal of Biotechnology*, 142:56–63.

Black, C.A. (1957). Soil-Plant Relationsships. John Wiley and Ons, Inc., Neywork.

Black, C.A. (1965). Methods of Soil Analysis Part 2, Amer. Society of Agronomy Inc., Publisher Madisson, Wilconsin, U.S.A., p:1372-1376.

Bouyoucos, G.J. (1955). A Recalibration of the Hydrometer Method for making mechanical analysis of the soils. *Agronomy Journal*, 4(9):434.

Bremner, J.M. (1965). Methods of soil analysis. American Society of Agronomy Inc., Medison, WT.

Chang, I.S., Zhao, J., Yin, X., Wu, J., Jia, Z., & Wang, L. (2011). Comprehensive utilizations of biogas in Inner Mongolia. *Renewable and Sustainable Energy Reviews*, 15:442-1453.

Chiew, Y.L., Spångberg, J., & Baky, A. (2015). Environmental impact of recycling digested food waste as a fertilizer in agriculture – A case study. *Resources, Conservation and Recycling*, 95:1-14.

Evliya, H. (1964) Kültür bitkilerinin beslenmesi. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Sayı:10, Ankara (in Turkish).

Ferdous, Z., Ullah, H., Datta, A., Anwar, M., & Ali, A. (2018). Yield and profitability of tomato as influenced by integrated application of synthetic fertilizer and biogas slurry. *International Journal of Vegetable Science*, 24(5):445-455.

Ferdous, Z., Ullah, H., Datta, A. Attia, A., Rakshit, A., & Molla, S.H. (2020). Application of biogas slurry in combination with chemical fertilizer enhances grain yield and profitability of maize (*Zea mays* L.). *Communications in Soil Science and Plant Analysis*, 51(19):2501-2510.

İbil, A. (2019). The effects of different solid and liquid biogas wastes on corn (*Zea mays* L.) yield and quality. Master Thesis. Adnan Menderes University, Aydın.

Jackson, M. (1967). Soil chemical analysis prentice. Hall of India Private Limited, New Delhi, 498(1).

Kacar, B. (1972). Bitki ve toprağın kimyasal analizleri. II. Bitki Analizleri, A.Ü. Ziraat Fak. Yayınları: 453, Ankara (in Turkish).

Kacar, B., & Inal, A. (2008). Bitki Analizleri. Nobel Yayın, 1241, 891 (in Turkish).

Karaman, R., & Türkay, C. (2022). Effect of biogas waste applications on agronomic and some quality traits in barley (*Hordeum vulgare* L.). *Journal of Agriculture Faculty of Ege University*, 59(4):633-643.

Knudsen, D., Peterson, G.A., & Pratt, P.F (1982). Lithium, sodium and potassium. Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties.) The GW (1982). Exchangeable cations. pp. 159-l65. Chemical and Microbiological Properties. Agronomy Monograph No.9 (2nd Ed). ASA-SSSA, Madison. Wisconsin, USA.

Koszel, M., & Lorencowicz, E. (2015). Agricultural use of biogas digestate as a replacement fertilizers. *Agriculture and Agricultural Science Procedia*, 7:119-124.

Koszel, M., Parafiniuk, S., Szparaga, A., Bochniak, A., Kocira, S., Atanasov, A.Z., & Kovalyshyn, S. (2020). Impact of digestate application as a fertilizer on the yield and quality of winter rape seed. *Agronomy*, 10:878.

Kouřimská L., Poustková I., & Babička L. (2012). The use of digestate as a replacement of mineral fertilizers for vegetables growing. *Scientia Agriculturae Bohemica,* 43(4):121-126.

Li, N., Yang, X., Liu, J., Liu, Y., Chen, Q., Wu, F., & Chang, R. (2023). Effect of raw material and application rate of biogas slurry on Cucumber growth, Fusarium wilt suppression, and soil properties. *Environmental Technology & Innovation*, 32:103396.

Liao, C.F. (1981). Devarda's alloy method for total nitrogen determination. *Soil Science Society of America Journal*, 45(5):852-855.

Lindsay, W.L., & Norvell, W. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3):421-428.

Losak, T., Hlusek, J., Zatloukalova, A., Musilova, L., Vitezova, M., Skarpa, P., Zlamalova, T., Fryc, J., Vitez, T., Marecek, J., & Martensson, A. (2014). Digestate from biogas plants is an attractive alternative to mineral fertilisation of kohlrabi. *Journal of Sustainable Development of Energy, Water and Environment Systems*, 2(4):309-318.

Lukehurst C.T., Frost, P., & AlSeadi, T. (2010). Utilisation of digestate from biogas plants as biofertiliser. *IEA Bioenergy*, 1-36.

Makadi, M., Tomocsik, A., Eichler-Loebermann, B., & Schiemenz, K. (2008). Nutrient cycling by using residues of bioenergy production—Effects of biogas-digestate on plant and soil parameters. *Cereal Research Communication*, 36:1807–1810.

Moller, K., Stinner, W., Deuker, A., & Leithold, G. (2008). Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. *Nutrient Cycling in Agroecosystems,* 82:209–32.

Nkoa, R. (2014). Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy for Sustainable Development*, 34:473-492.

Olsen, S.R., & Sommers, E.L. (1982). Phosphorus Availability Indices. Phosphorus Soluble in Sodium Bicarbonat Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Editors: A.L. Page. R.H. Miller. D.R. Keeney, 404-430.

Ronga, D., Setti, L., Salvarani, C., De Leo, R., Bedin, E., Pulvirenti, A., Milc, J., Pecchioni, N., & Francia, E. (2019). Effects of solid and liquid digestate for hydroponic baby leaf lettuce (*Lactuca sativa* L.) cultivation. *Scientia Horticulturae*, 244:172–181.

Rózyło, K., Swieca, M., Gawlik-Dziki, U., Andruszczak, S., Kwieci´nska-Poppe, P., & Kraska, P. (2017). Phytochemical properties and heavy metal accumulation in wheat grain after three years fertilisation with biogas digestate and mineral waste. *Agricultural and Food Science*, 26:148-159.

Soltanpour, P.N., & Workman, S.M. (1981). Use of inductively-coupled plasma spectroscopy for the simultaneous determination of macro and micronutrients in NH_4HCO_3-DTPA extracts of soils. In Barnes R.M. (ed). Developments in Atomic Plasma Analysis, USA, pp. 673-680.

Weiland, P. (2010). Biogas production: current state and perspectives. *Applied Microbiology and Biotechnology*, 85:849-860.

Xu, C., Tian, Y., Sun, Y., & Dong, L. (2013). Effects of biogas slurry irrigation on growth, photosynthesis, and

nutrient status of Perilla frutescens seedlings. *Communications in Soil Science and Plant Analysis*, 44(22):3381-3390.

Yadav, A., & Garg, V.K. (2016). Vermiconversion of biogas plant slurry and parthenium weed mixture to manure. *International Journal of Recycling of Organic Waste in Agriculture*, 5:301-309.

Yaraşır, N., Erenkul, O., & Yiğit, A. (2018). The effect of different doses of liquid biogas fermentation wastes on yield and quality of bread wheat (*Triticum aestivum* L.). *Adnan Menderes University Faculty of Agriculture Journal of Agricultural Sciences,* 15(2):9-16.

Yaylacı, C., & Erdal, İ. (2021). Effect of biogas digestate, incubation, and npk fertilization on some soil properties and growth, mineral nutrition, and nutrient uptake of wheat. *Journal of Elementology*, 26(3):697-715.

Yu, F.B., Luo, X.P., Song, C.F., Zhang, M.X., & Shan, D.S. (2010). Concentrated biogas slurry enhanced soil fertility and maize quality. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science,* 60:262–68.

Yurtsever, N. (1984). Deneysel istatistik metotları. Köy Hizmetleri Genel Müdürlüğü Yayınları, No: 56, Ankara (in Turkish).

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RESEARCH PAPER

HORTICULTURAL STUDIES

Identification of Quality Characteristics of Autochthonous Karasüt Apple during Cold Storage

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Abstract

The main objective of the research was to assess weight loss, respiration rate, firmness, soluble solids content (SSC), titratable acidity and vitamin C content of the autochthonous Karasüt apple throughout cold storage. The 'Granny Smith' cultivar was used as positive control. The fruit was kept at a temperature of 0.0±0.5°C and relative humidity of 90±5%. Quality losses were observed in the apples during cold storage. The weight loss of Karasüt apple (6.70%) was higher than that of Granny Smith (2.20%) at the end of cold storage. A lower respiration rate was measured in the Karasüt apple (1.23 nmol CO_2 kg^{-1} s^{-1}), compared to the positive control (1.53 nmol CO_2 kg^{-1} s^{-1}) at harvest. On the contrary, the respiration rate was higher on days 30 and 60. The fruit firmness of Granny Smith (27.14 N) was higher than that of Karasüt (22.47 N) at the end of cold storage. During the cold storage, a higher SSC was obtained from Karasüt apple compared to the positive control. However, titratable acidity was lower in Karasüt apple. In the first two measurements of cold storage, the vitamin C of Karasüt apple was higher than that of the positive control. As a result, it was revealed that Karasüt apple had faster quality losses during cold storage than Granny Smith apple cultivar.

1. Introduction

Apple, a pome fruit, can be grown in a very wide range of climates worldwide, including temperate, tropical, and subtropical regions. It is the fourth most widely produced fruit globally, following citrus, grapes, and bananas (Palmer et al., 2003). Among temperate climate fruit species, it located the first place in terms of production. Türkiye produces approximately 4.5 million tons (about 5%) of the 93.14 million tons of apples produced worldwide, ranking second after China in terms of production volume (FAO, 2023).

Apple is consumed not only fresh but also in various processed forms, such as fruit juice, puree, concentrate, as filling for pastries, in sauces, wines, and as dried fruit. Due to its richness in vitamins, minerals, and especially phenolic compounds like flavonoids, along with their antioxidant properties, apples are highly favored by consumers (Boyer and Liu, 2004). The widespread cultivation of apples and their long storage period allow consumers easy access to this fruit (Palmer et al., 2003). Naturally, the extensive cultivation and production of apples across different regions lead to the utilization of numerous standard and autochthonous cultivars to match consumers' preferences. Autochthonous varieties specific to certain regions are consumed more than commercial varieties in their respective areas. Moreover, these autochthonous varieties often receive a "Geographical Indication" certification, enabling them to be sold at higher prices, thus providing higher profits to the producers. Additionally, this recognition contributes

significantly to promoting the region and serves as a source of pride and motivation for the autochthonous producers (Şahin, 2013).

The main aim of this research was to determine the weight loss and changes in certain quality characteristics of the locally grown 'Karasüt' apple during cold storage in the Bulancak district of Giresun province, Türkiye. With the first data obtained from this study, it is aimed to obtain a geographical indication designation for the autochthonous 'Karasüt' apple.

2. Material and Methods

In the study, autochthonous Karasüt apple variety and Granny Smith cultivar (*Malus domestica* Borkh.) were used as plant materials (Figure 1). Fruit were manually harvested at the commercial ripening stage from a producer orchard in Bulancak district of Giresun province, Türkiye. Harvesting for Karasüt and Granny Smith apples took place approximately 160 and 170 days after full bloom, respectively. The Granny Smith apple trees, aged 10 years, were grafted onto MM111 rootstocks, while the 20-year-old Karasüt apple trees were grafted onto seedling rootstocks.

The fruit were placed into 5 kg plastic crates and transported to the laboratory using refrigerated vehicles at [15±0.5°C and 85±5% relative humidity (RH)]. Damaged and diseased fruit were removed and discarded. The remaining fruit were distributed in the plastic crates, with approximately 20 fruit per crate to represent each replication. A total of 12 crates were prepared for each variety. Subsequently, the fruit were subjected to precooling with cold air at +4±0.5°C and 90±5% RH for 24 hours. Following precooling, the fruit were stored at 0.0±0.5°C and 90±5% RH for a period of 120 days, during which measurements and analyses were conducted at monthly intervals (30, 60, 90, and 120 days). Three crates were taken for each measurement period, and each crate represented one replication (3 rep).

2.1. Weight loss, respiration rate and fruit flesh firmness

For each replication of Karasüt and Granny Smith apples, fruit in the crates were weighed on a digital scale with a precision of 0.01 g (Radwag, Poland) before storage. Subsequently, the same crates were weighed again during each measurement period (measurements were conducted inside the cold storage). Based on the initial and post-storage measurements, the weight loss was calculated as a percentage (%) (Ozturk et al., 2017).

For respiration rate measurements, 3 fruits were taken from each replication. They were placed in a gas-tight 2-liter container, and after 1 h, the CO_2 concentration in the environment was measured using a digital carbon dioxide gas analyzer (Vernier, Oregon, USA) with a typical accuracy of 5.0%). The measured values were then determined based on the volume and weight of the fruit inside the container and presented as nmol $CO_2\,kg^{-1}\,s^{-1}$ (Ozturk et al., 2022).

Measurements for fruit firmness, 10 fruits were used in each replication. Measurements were made at 3 different points (at an angle of 120 degrees between them at the equator) on the equatorial part. Fruits' peel was excised using a cutter, and subsequently, measurements were taken using the 11.1 mm tip of an Effegi penetrometer (FT-327, McCormick, USA). The obtained results were presented in N (Newton) (Ozturk et al., 2017).

2.2. Titratable acidity, vitamin C, soluble solids content

Initially, five fruit from each replication were washed with tap water, followed by rinsing with distilled water. A slice of approximately 3-4 cm width

Figure 1. Image of studied apple varieties.

was cut from each fruit using a stainless steel knife, and the peels were removed. The slices were then pureed in a blender (Philips, Türkiye), and the obtained homogenate was filtred through a cloth to obtain fruit juice. Soluble solids content (SSC) measurement in the fruit juice was carried out using a digital refractometer (PAL-1, USA), and the values were presented as a percentage (%). For titratable acidity, a sufficient amount of fruit juice was taken and diluted with distilled water. The pH of the solution was titrated with 0.1 N sodium hydroxide until it reached 8.2. The acidity was determined based on the amount of sodium hydroxide consumed, and the values were presented as % malic acid (Ozturk et al., 2017). For vitamin C measurement, approximately 10 mL of fruit juice was taken, and the method described by Karakaya et al. (2020) was employed using a digital reflectometer (Merck RQflex plus 10, Germany). The obtained values were presented as mg 100 g^{-1}.

2.3. Statistical analysis

In the study, data were subjected to normal distribution testing using the Kolmogorov-Smirnov Test. Homogeneity of variance was assessed using the Levene test. Descriptive statistics for the data meeting the assumptions were calculated, and analysis of variance (ANOVA) was performed for evaluation. Following the analysis of variance, the significance level among varieties was determined using the Tukey multiple comparison test (α=5%). The data obtained from the study were analyzed using IBM SPSS version 20.0 statistical analysis program.

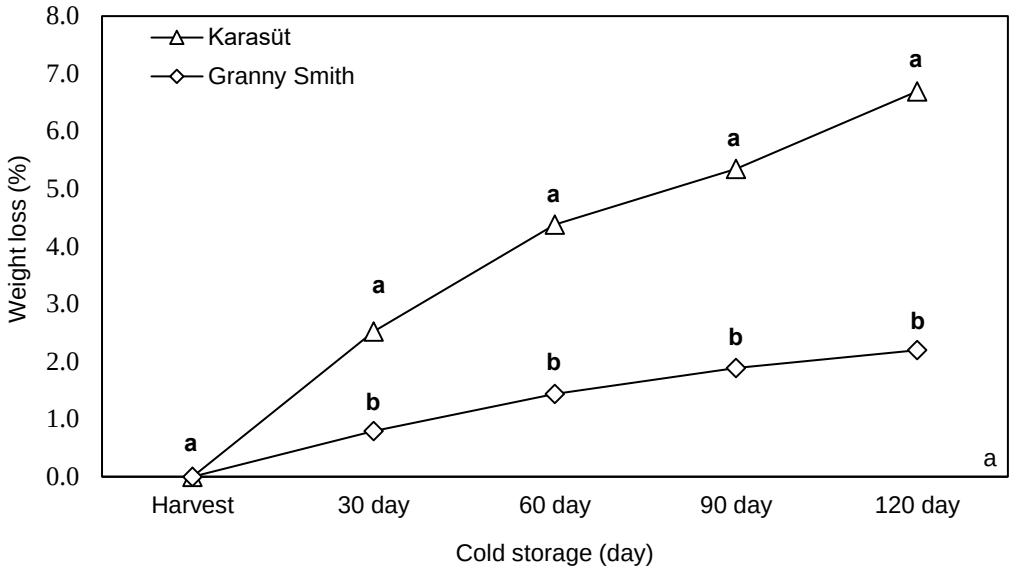
3. Results and discussion

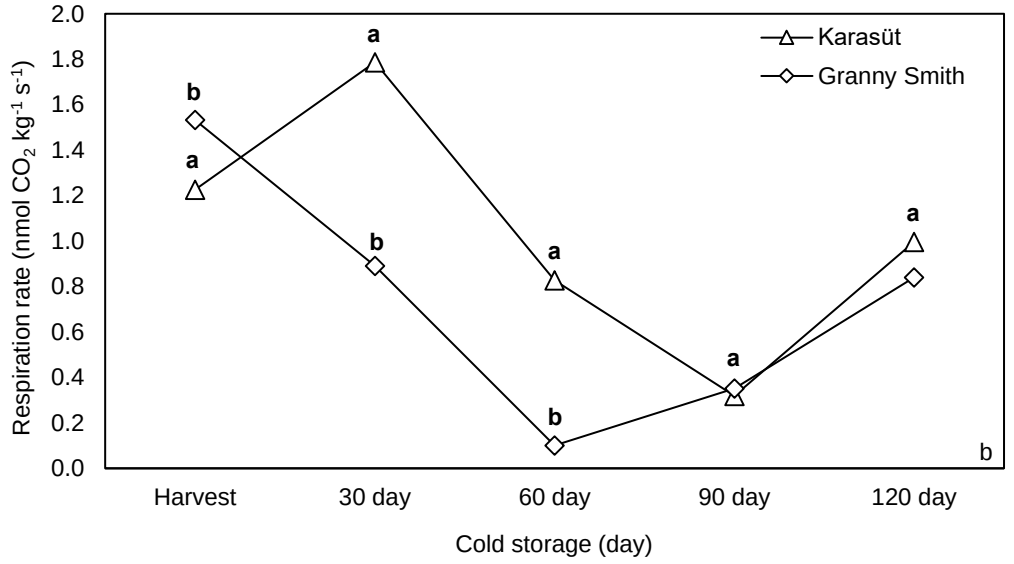
In the study, it was observed that throughout all measurement periods, the weight loss of Karasüt apples during cold storage was higher than that of Granny Smith apples. Conversely, Karasüt apples exhibited lower firmness compared to Granny Smith apples during all measurement periods. At the end of the storage period, the weight loss in Karasüt apples (6.69%) was approximately 3 times higher than that in Granny Smith apples (2.20%). On the 30^{th} and 60^{th}-day, a significant difference ($p<0.05$) in respiration rate was detected between Karasüt and Granny Smith apples. While Karasüt apples exhibited lower respiration rates than Granny Smith apples at harvest, significantly lower respiration rates were obtained during the first two measurements of the storage period (Figure 2).

After harvest, weight loss in fresh fruits and vegetables is an expected occurrence. However, the extent of this loss directly affects storage duration and fruit quality. Therefore, minimizing weight and firmness loss is desired. Of course, during the weight loss period, texture deterioration and softening of the fruit occur. In our study, the measured weight and firmness loss varied among varieties. In Karasüt apples, both weight and firmness loss were higher during cold storage. Indeed, researchers (Ghafir, 2009; Reig et al., 2017) have reported that weight and firmness loss may vary depending on the cultivar. Additionally, in apple varieties, the respiration rate in Karasüt apples was higher than that in Granny Smith apples during the first two measurements of storage. This higher respiration rate in Karasüt apples may have resulted in the greater weight and firmness loss. Simultaneously, the hydrolysis of cell wall polysaccharides occurs as starch breaks down, leading to rapid softening of the fruit (Jan and Rab, 2012; Ren et al., 2020). Moreover, variations in pectin content among different varieties can contribute to differences in firmness levels (Billy et al., 2008).

During harvest and cold storage, it was observed that the SSC values of Karasüt apples were significantly higher than those of Granny Smith apples. In contrast, the titratable acidity values were found to be lower. Karasüt apples exhibited a 42% higher SSC than Granny Smith apples at harvest. However, at the end of storage, Karasüt apples had a 33% higher SSC compared to Granny Smith apples. At harvest, the acidity of Granny Smith apples (1.79%) was 3 times higher than that of Karasüt apples (0.57%). However, at the end of storage, the measured acidity value in Granny Smith apples was 4.67 times that of Karasüt apples. Regarding vitamin C, significant differences ($p<0.05$) were observed between apples during both harvest and the 30^{th} and 60^{th} days of storage. Both at harvest and during the first two measurement periods of storage, Karasüt apples had significantly higher vitamin C content than Granny Smith apples. Throughout the storage period, a higher vitamin C loss was observed in Karasüt apples (Figure 3).

With ripening, starch in the apple is broken down and converted into sugars. SSC, which provides information about taste during consumption, increases with the progress of ripening, while acidity decreases. The breakdown of starch into sugars is among the fundamental reasons for the increase in SSC in fruit (Burdon et al., 2016). In the study, higher SSC and lower acidity were measured in Karasüt apples compared to Granny Smith apples during cold storage. Furthermore, variations in vitamin C content were observed between the varieties during harvest and the first two measurements of storage. Additionally, a decrease in vitamin C content was observed in both varieties during storage. Indeed, Hayat et al. (2005) reported that there may be potential decreases in vitamin C content during storage. Additionally, Nour et al. (2010) reported differences in vitamin C content among apple cultivars. In parallel with our findings, Jan and Rab (2012) stated that the apple cultivars (Royal Gala, Mondial Gala, Golden Delicious and Red Delicious) they examined were different in





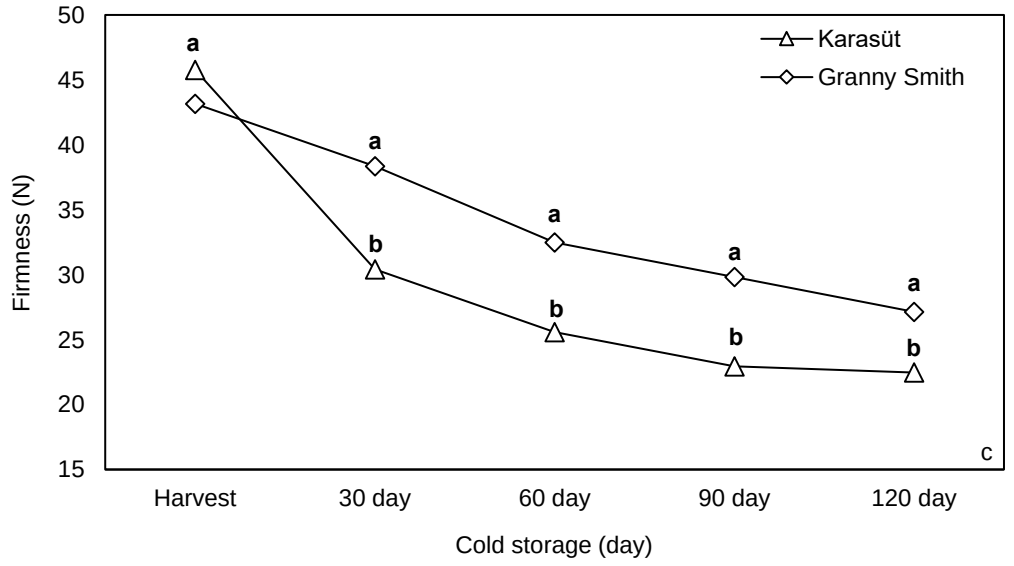


Figure 2. Weight loss (a), respiration rate (b) and firmness (c) of autochthonous Karasüt variety and Granny Smith cultivar apples during cold storage (Means indicated with same lower-case letter vertically didn't significant, Tukey's test, $p<0.05$).

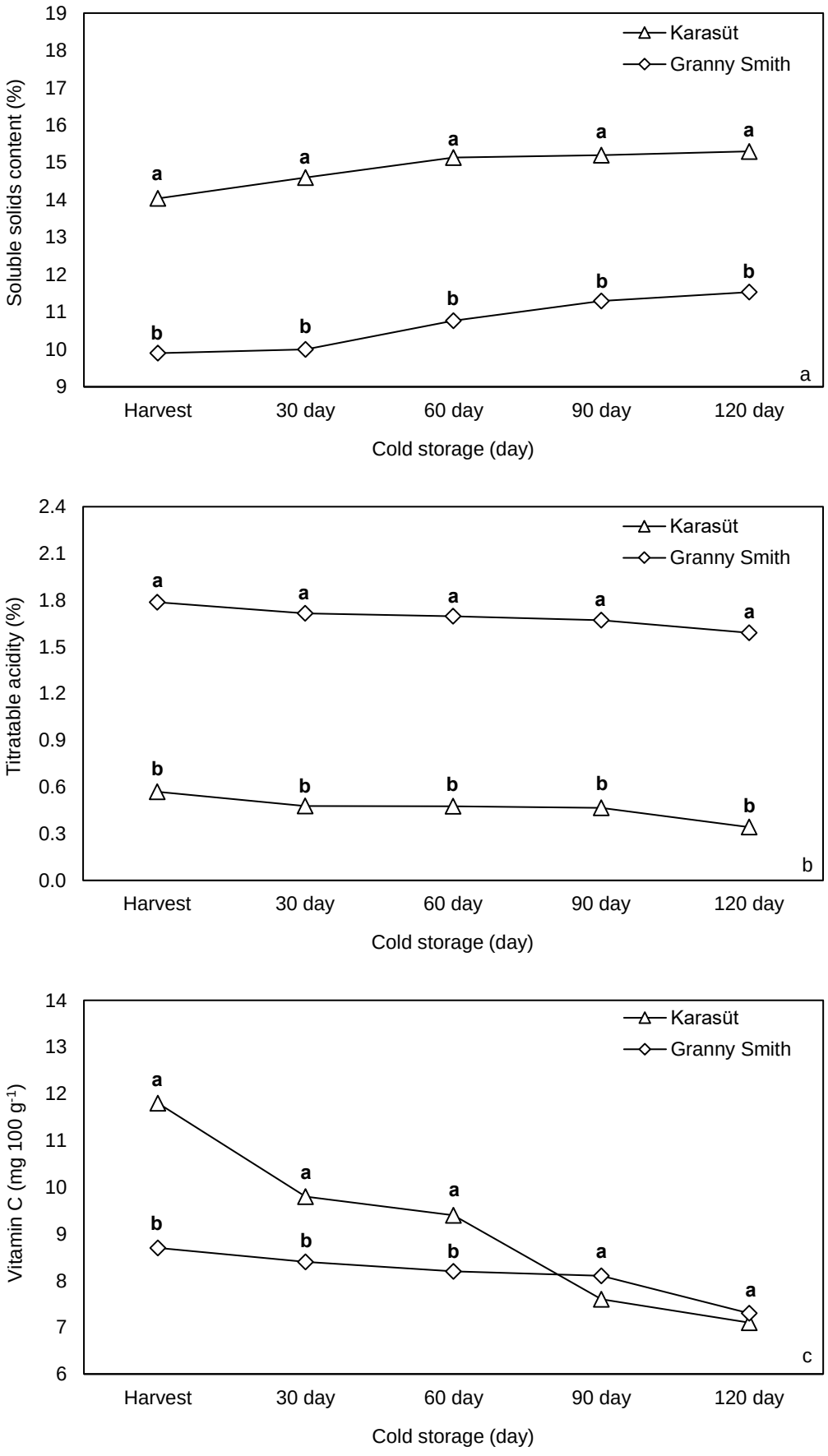


Figure 3. Soluble solids content (a), titratable acidity (b) and vitamin C (c) of autochthonous Karasüt variety and Granny Smith cultivar apples during cold storage (Means indicated with same lower-case letter vertically didn't significant, Tukey's test, $p<0.05$).

vitamin C, SSC and acidity contents at harvest and during storage. Furthermore, some researchers have indicated that certain factors such as higher storage temperature, injuries, low relative humidity, and chilling damage during storage may lead to a decrease in vitamin C content (Nour et al., 2010; Mditshwa et al., 2017).

4. Conclusion

As a result, during storage, all fruit experience quality losses. However, the extent of these quality losses is significant consumer acceptance. This study revealed that Karasüt apple had higher weight and firmness losses compared to Granny Smith apple. At the end of cold storage, the weight loss of the Karasüt apple was approximately 3 times greater than that of the Granny Smith cultivars. The flesh firmness of the Karasüt apple was 17% lower than that of the Granny Smith apple. Additionally, the vitamin C loss in Karasüt apple was more pronounced during cold storage.

References

Billy, L., Mehinagic, E., Royer, G., Renard, C.M., Arvisenet, G., Prost, C., & Jourjon, F. (2008). Relationship between texture and pectin composition of two apple cultivars during storage. *Postharvest Biology and Technology*, 47(3):315-324.

Boyer, J., & R.H. Liu. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal,* 3:5.

Burdon, J., Pidakala, P., Martin, P., Billing, D., & Boldingh, H. (2016). Fruit maturation and the soluble solids harvest index for 'Hayward' kiwifruit. *Scientia Horticulturae*, 213, 193-198.

FAO (2023). https://www.fao.org/faostat/en/#data/QCL (Accessed date: 17 July 2023).

Ghafir, S.A. (2009). Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions. *Acta Biologica Szegediensis*, 53(1):21-26.

Hayat, I., Masud, T., & Rathore, H.A. (2005). Effect of coating and wrapping materials on the shelf life of apple (*Malus domestica* cv. Borkh). *International Journal of Food Safety*, 5:24-34.

Jan, I., & Rab, A. (2012). Influence of storage duration on physico-chemical changes in fruit of apple cultivars. *Journal of Animal and Plant Sciences*, *22*(3):708-714.

Karakaya, O., Aglar, E., Ozturk, B., Gun, S., Ates, U., & Ocalan, O.N. (2020). Changes of quality traits and phytochemical components of jujube fruit treated with preharvest GA_3 and Parka during cold storage. *Turkish Journal of Food and Agriculture Sciences*, 2(2):30-37.

Mditshwa, A., Magwaza, L. S., Tesfay, S. Z., & Opara, U. L. (2017). Postharvest factors affecting vitamin C content of citrus fruits: A review. *Scientia Horticulturae*, 218:95-104.

Nour, V., Trandafir, I., & Ionica, M.E. (2010). Compositional characteristics of fruits of several apple (Malus domestica Borkh.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(3):228-233.

Ozturk, B., Yıldız, K., Uzun, S., & Ozturk, A. (2017). Effects of pre-harvest AVG treatments on fruit quality of Jonagold apple cultivar throughout cold storage. *International Journal of Agriculture and Wildlife Science*, 3(1):1–5.

Ozturk, A., Ozturk, B., Karakaya, O., Gün, S., Ates, U., Yıldız, K., & Gundogdu, M. (2022). Preliminary results: Combination of MAP and Aloe vera gel in medlar fruit. *Erwerbs-Obstbau*, 64(1):37-45.

Palmer, J.W., Prive, J.P., & Tustin, D.S. (2003). Temperature. In: Ferree, D.C., Warrington, I.J. (Eds.), Apples: Botany, Production and Uses. Cabi publishing, pp. 217–236.

Reig, G., Alegre, S., Cantín, C. M., Gatius, F., Puy, J., & Iglesias, I. (2017). Tree ripening and postharvest firmness loss of eleven commercial nectarine cultivars under Mediterranean conditions. *Scientia Horticulturae,* 219:335-343.

Ren, Y. Y., Sun, P. P., Wang, X. X., & Zhu, Z. Y. (2020). Degradation of cell wall polysaccharides and change of related enzyme activities with fruit softening in Annona squamosa during storage. *Postharvest Biology and Technology*, 166:111203.

Şahin, G. (2013). Importance of the geographical indications and geographical indications of Vize (Kirklareli). *Pamukkale Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*, (15), 23-37 (in Turkish).