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Effect of volk oil and potassium nitrate on budbreak, yield and some quantitative and qualitative characteristics of pistachio (*Pistacia vera* L.) nuts

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ABSTRACT

Purpose: The aim of this study was to improve yield, and characteristics of pistachio nuts of three cultivars by foliar application of volk oil and potassium nitrate. **Research method**: The study was carried out during the bud swelling stage using a factorial design, following a randomized complete block design with four replications. The experimental factors consisted of three cultivars, namely 'Fandoghi', 'Hasan-Abbasi', and 'Haj-Abdollahi', and three treatments: volk oil (1% and 2%), potassium nitrate (KNO_3) (0.3% and 0.5%), and a mixture of volk oil and KNO3 (1% volk oil + 0.3% KNO_3, and 1% volk oil + 0.5% KNO_3). A control treatment was also included in the study. **Findings:** The results of this study showed that the highest percentage of open shell nuts (52.17%) was observed in 'Hasan-Abbasi' cultivar, and the highest fresh weight of 100 nuts in 'Haj-Abdollahi' (202.2 g). The lowest ounce (29.07), the highest dry weight of 100 nuts and 10 kernels weight were obtained 76.26 and 26.56 g in 'Fandoghi' cultivar, respectively. The use of 2% volk oil treatment was effective in budbreaking, as well as increasing the percentage of open shell nuts and reducing the percentage of balk nuts. **Research limitations:** There was no limitation. **Originality/Value:** 0.5 % KNO_3 increased the fresh and dry weight of 100 nuts and decreased ounce of pistachio in all cultivars. Therefore, the use of volk oil and KNO_3 treatments can be effective in pistachio orchards, and increase the quantity and quality of the yield.

INTRODUCTION

Pistachio (*Pistacia vera* L.) is a dioecious tree belonging to the Anacardiaceae family (Al-Saghir, 2010). It is one of the most important horticultural products and is compatible with the salty regions and arid and semi-arid climatic conditions of Iran, which is one of the richest sources of pistachios in the world (Akbari, 2015; Azarmi-Atajan & Sayyari-Zohan, 2022). The US, Turkey, and Iran produce 97% of the world's pistachio (I.N. & D. Nuts & Dried Fruits Statistical Yearbook, 2021). In recent years, the global temperature has increased abnormally, which is the result of the greenhouse phenomenon and global warming, and has affected human activities, especially the agricultural sector. Recent reports from agricultural meteorological authorities and horticulturists have indicated the effectiveness of these changes following the damage of providing chilling requirements and shifting the date of the phenological stages. Horticulture is one of the most important sectors in agriculture, and global climate changes with the average change and variance of meteorological data, especially its warming, increase the possibility of serious events, which can have major consequences in the annual cycle of orchards. An increase in temperature in the future affected by climate change may disrupt the process of meeting the chilling requirements of the winter season and affect the production of orchard products (Bhatti et al., 2006;).

Pistachios require dry summers and cold winters, and if the winter is warm, the budbreak is delayed. Pistachio tolerates heat to +40 and cold to -18. The chilling requirement of pistachio cultivars is between 600 hours in 'Kaleh- Ghochi' and 1200 hours in 'Ghafouri' cultivar (Akbari et al., 2015; Beriner et al., 1985). Rahemi and Pakkish (2009) investigated the chilling requirement of pistachio in 'Kaleh- Ghochi', 'Ahmad-Aghaei', 'Owhadi' and 'Akbari' cultivars and found that 'Kaleh- Ghochi' with a chilling requirement of 750-800 hours has the lowest cold requirement and 'Akbari' had the highest chilling requirement with 1200 hours in both years.

Many chemicals have remedial effects on dormancy, but few have been found to be suitable for use in field conditions. Chemical substances that have been studied in different countries include mineral oils (volk oil), potassium nitrate (Khayyat et al., 2010), thiourea, and cyanamides, which are used as nutritional supplements and agents for dormancy breaking (Lamont et al., 1987). Horticultural mineral oils are widely used to control the blossoming of buds in apples, pears, peaches, and apricot trees. In addition, the use of volk oil in winter accelerates flowering, enhances the uniformity of flowering, and increases the quality and quantity of pistachios (Beede & Padillia, 1998).

Some studies have also been conducted on the effects of these materials on pistachio chilling requirements. In a study, it was shown that the use of volk oil, soybean oil, and fatty acids makes the trees go to the flowering stage faster in 2-4 days (Nazouri, 2007). Rahemi and Asghari (2004) studied the effects of volk oil, hydrogen cyanamide, and potassium nitrate on the cultivar 'Ahmad-Aghaei' and found that the use of these substances increased the yield, open shell nuts, and decreased the percentage of blank nuts. The results of a study investigating the effect of foliar spraying with volk oil showed that this treatment increased the fresh weight of 'Qazvini' and 'Owhadi' cultivars, and other characteristics such as blanks, as well as the dry weight of the whole fruit (Kashanizadeh, 2006).

Ghrab et al. (2014) studied the use of cyanamide hydrogen and its effect on breaking bud dormancy, flowering, and performance of pistachio trees in hot regions. The results showed that 4% hydrogen cyanamide broke the natural dormancy and caused the simultaneous flowering of male and female trees, which increased the pollination of trees. In addition, the results showed that the growth of stem and leaf surfaces and starch were affected by the use of

this substance. The application of cyanamide 45 days before budbreak improved crop production and prevented anomalies in the lack of chilling requirement.

Due to the global warming and the lack of chilling requirements of pistachio trees, a study was conducted with the aim of investigating the chilling requirements and the effect of Volk oil on the vegetative and reproductive characteristics of pistachio trees of 'Owhadi' and 'Akbari' cultivars. The results showed that this treatment was very effective in improving the measured characteristics (Mahmoudi et al., 2022).

The climate of the Yazd Province is hot and dry. Pistachio trees grown in this province often do not meet chilling requirements. Therefore, the purpose of this research is to investigate the effect of foliar spraying with volk oil and potassium nitrate on bud break, yield and characteristics of pistachio nuts of the cultivars Fandoghi', 'Hasan-Abbasi' and 'Haj-Abdollahi'.

MATERIALS AND METHODS

This research was conducted in a 15-year-old pistachio orchard in Ardakan County (32.20° N, 53.48° E), Yazd Province, Iran, during 2015. The three selected cultivars were: 'Fandoghi', 'Hasan-Abbasi' and 'Haj-Abdollahi'. The distance between the trees was 4 × 5 m, and trees were irrigated using flood irrigation. The soil characteristics are shown in Table 1. The annual fertilizer applied was 450 kg ammonium sulfate, 6 kg iron, and 70 kg calcium per hectare. The experiment was conducted concurrently with the bud swelling stage, which typically occurs in early March. It followed a factorial design within a randomized complete block, comprising four replications. Additionally, three chemical treatments were applied, including volk oil (1% and 2%), potassium nitrate (KNO_3) (0.3% and 0.5%), as well as a combination of volk oil and KNO_3 at concentrations of 1% volk oil + 0.3% KNO_3 and 1% volk oil + 0.5% KNO_3. A control treatment (water) was also included in the experimental design.

The desired factors were evaluated after treatment application. The date of beginning of flowering and leaf formation were measured. Fruit harvesting was performed in summer from four uniform branches in four directions of the tree that were marked, and then all measurements, such as percentage of blank, and open shell nuts, fresh and dry weight of 100 nuts, weight of 10 kernels, ounce, cluster weight, nuts per clusters, and number of open shell and blank nuts in the cluster, were measured. To determine the ounce, the number of pistachio nuts in 142 g was divided by five, and the resulting number shows the ounce of the pistachio. In addition, the number of pistachios in 28.4 grams can be used as the basis for an ounce of pistachios (Mirabzadeh Ardakani et al., 2021).

Data analysis

Data were analyzed using the procedure for analysis of variance (ANOVA) of the SAS (ver. 9.1), and the mean comparison of data was performed based on Duncan's test (5%).

Table 1. Physicochemical characteristics of the experimental soil during the experiment season.

Parameters	Clay (%)	Silt (%)	Sand (%)	Soil texture	pH	EC (ms/cm)	Organic Carbon (OC) %	$CaSO_4$ (%)	N (%)	P (ppm)	K (ppm)	Na (meq/L)	Mg (meq/L)	Ca (meq/L)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
Amount	9	36	55	Sandy loam	7.7	8.1	0.456	22.48	0.039	7.2	589.4	45.5	21.4	29.6	0.58	1.06	3.4	2.4

RESULTS AND DISCUSSION

Vegetative and reproductive budbreak time

The results showed that 'Haj-Abdollahi' cultivar had flowering and leafing later than other cultivars. Among the treatments, the 2% volk oil treatment accelerated flowering and leafing, and the control treatment had the longest flowering and leafing (Table 2). In relation to cluster formation, foliar spraying of trees with chemical treatments caused the uniformity in fruit formation in Hasan-Abassi, 'Haj-Abdollahi',' and 'Fandoghi' cultivars compared with the control treatment. In addition, foliar spraying caused the budbreak of more reproductive buds in the treated trees than in the control treatment (without treatments).

Insufficient annual chilling of fruit trees, especially pistachios, is a major problem observed in many regions with warm winters (Eskandari Torbaghan, 2023). In winter, the plant is in the dormancy stage, which does not grow and develop even if it is placed in a suitable environment. The dormancy period was activated by a decrease in temperature and daylight. However, getting out of it requires chilling, so without this stage, the plant's annual cycle is not completed and the plant will not be able to grow naturally (Talaie et al., 2006).

The results of this research showed that the use of mineral oils such as 1% volk oil and its combination with 0.3 and 0.5 % potassium nitrate accelerated uniform flowering (Table 2), which was similar to the results of Beede and Ferguson (2001), who stated that volk oil is effective in dormancy breaking. The effect of volk oil in breaking the dormancy is due to the reaction of the plant to a moderate stress, in which case the plant increases its metabolism for breathing, so that it can break down the oil, and this increase in activity causes the early growth of buds. In this study, the best treatment for early flowering was 2% volk oil treatment, which increased the risk of spring frost. Nazouri (2007) showed that the use of volk oil, soybean oil, and fatty acids makes the trees go to the flowering stage faster by 2-4 days. In addition, the use of oils had a positive effect on the germination of pollen grains in male pistachio trees. The results of this study showed that potassium nitrate alone did not play a role in flowering and early leafing and caused a delay in flowering and fruit formation, but caused the uniformity of fruit formation, and using volk oil with potassium nitrate in cultivars caused coordination in cluster formation and uniform cluster formation. The results also showed that foliar spraying caused the reproductive buds of the trees to open compared with the control treatment. Trees that were not treated had fewer reproductive buds, which are consistent with the results of previous studies (Asghari, 2002, Javanshah & Esmaeilizadeh, 2004).

Table 2. Interaction effects of treatments and cultivars on the beginning time of flowering and leafing.

Cultivars	Fandoghi		Hasan-Abbasi		Haj-Abdollahi	
Treatments	Beginning of flowering (day)	Beginning of leafing (day)	Beginning of flowering (day)	Beginning of leafing (day)	Beginning of flowering (day)	Beginning of leafing (day)
Control	18b	21b	18b	20c	19a	22a
Volk oil (1%)	13g	16f	14f	16f	15e	18d
Volk oil (2%)	12h	15g	12h	14h	14f	17e
KNO_3 (0.3%)	17c	20c	16d	18d	17c	20c
KNO_3 (0.5%)	18b	21b	18b	20c	17c	20c
Volk oil (1%) + KNO_3 (0.3%)	14f	17e	14f	16f	15e	18d
Volk oil (1%) + KNO_3 (0.5%)	14f	17e	14f	16f	15e	18d

Means followed by different letters in each column indicate significant differences at $p < 0.05$ (Dunkan test).

Table 3. Interaction effects of treatments and cultivars on open shell and blank nuts.

Cultivars	Fandoghi		Hasan-Abbasi		Haj-Abdollahi	
Treatments	Open shell (%)	Blank (%)	Open shell (%)	Blank (%)	Open shell (%)	Blank (%)
Control	41 ij	28 ab	39.25i	29.75a	38.75j	29.5a
Volk oil (1%)	50.75 cde	23 defg	55.5b	25.25bcd	45gh	26.5bc
Volk oil (2%)	62.5 a	17 j	65a	21.25fgh	51.5cde	21fgh
KNO_3 (0.3%)	43.5 hi	23.5def	49.25def	22efgh	46.5fgh	23.5def
KNO_3 (0.5%)	48 efg	22 efgh	53bcd	20ghi	53.25bc	25cde
Volk oil (1%) + KNO_3 (0.3%)	52.5bcd	22.5 defg	51.75bcd	21fgh	52bcd	17.75ij
Volk oil (1%) + KNO_3 (0.5%)	52.25bcd	22.5 defg	51.5cde	20.5fghi	54.5bc	19.25hij

Means followed by different letters in each column indicate significant difference at $p < 0.05$ (Dunkan test).

Nuts characteristics

The results of this research showed that the highest open shell nuts was obtained with 2% volk oil treatment in 'Hasan-Abbasi' cultivar (65%) and 'Fandoghi' cultivar (62.5%), which had a significant difference with other treatments. Also, the lowest open shell nuts was obtained with the control treatment of 'Fandoghi', 'Hasan-Abbasi' and 'Haj-Abdollahi' cultivars, 41%, 39.25%, and 38.75%, respectively, which had significant differences in other treatments as well (Table 3).

The highest percentages of blank nuts in the control treatments were 28% for 'Fandoghi', 29.5% for 'Haj-Abdollahi' and 29.75% for 'Hasan-Abbasi'. Also, the lowest percentage of blank nuts was obtained in the treatment of 2% volk oil for 'Fandoghi' (17%), and with the combined treatments of 1% volk oil with 0.3 and 0.5% potassium nitrate for 'Haj-Abdollahi' cultivar (19.25 and 17.75%, respectively) (Table 3).

The maximum fresh weight of 100 nuts was obtained in the treatment of 0.5 % potassium nitrate for 'Haj-Abdollahi' cultivar (260.32 g) and 0.3 and 0.5 % potassium nitrate for 'Fandoghi' cultivar, (223.02 g and 230.32 g, respectively) which was significantly different from that of the other treatments. The lowest fresh weight of 100 nuts was obtained in the control and volk oil and potassium nitrate combined treatments (Table 4).

Based on the results of this research, the maximum dry weight of 100 nuts was obtained in the potassium nitrate treatment in the cultivars of 'Fandoghi' and 'Haj-Abdollahi', (88.15 and 95.85 g, respectively). Also, the lowest dry weight of 100 nuts was observed in the control treatments of 'Haj-Abdollahi' and 'Hasan-Abbasi' cultivars (61.5 and 54.5 g, respectively) (Table 4).

Table 4. Interaction effects of treatments and cultivars on fresh and dry weight of 100 nuts.

Cultivars	Fandoghi		Hasan-Abbasi		Haj-Abdollahi	
Treatments	Fresh weight of 100 nuts (%)	Dry weight of 100 nuts (%)	Fresh weight of 100 nuts (%)	Dry weight of 100 nuts (%)	Fresh weight of 100 nuts (%)	Dry weight of 100 nuts (%)
Control	172.62hi	72.55cd	163.85i	61.5e	164.8i	54.5f
Volk oil (1%)	187.75defgh	71.85d	178.52efghi	72.92cd	206.9c	74.62cd
Volk oil (2%)	191.075def	78.97c	172.57hi	72.75cd	208.82c	74.62cd
KNO_3 (0.3%)	223.025b	75.4cd	184.45efgh	73.55cd	183.05efgh	74.15cd
KNO_3 (0.5%)	230.32b	95.85a	193.75cde	75.27cd	260.325a	88.15b
Volk oil (1%) + KNO_3 (0.3%)	178.05efghi	71.35d	176.17fghi	69.87d	201.77cd	75.2cd
Volk oil (1%) + KNO_3 (0.5%)	176.67fghi	69.25d	174.27ghi	69.47d	189.75defg	72.32cd

Means followed by different letters in each column indicate significant difference at $p < 0.05$ (Dunkan test).

Table 5. Interaction effects of treatments and cultivars on ounce and weight of 10 kernels.

Cultivars	Fandoghi		Hasan-Abbasi		Haj-Abdollahi	
Treatments	Ounce	Weight of 10 kernels (g)	Ounce	Weight of 10 kernels (g)	Ounce	Weight of 10 kernels (g)
Control	32 cdef	4.8fgh	33bcd	4.15i	37a	3.45j
Volk oil (1%)	28.5 h	5.32bcd	33.5bc	4.95defg	31.25def	4.82efgh
Volk oil (2%)	26.5 i	5.5bc	31.5def	4.52ghi	28.5h	4.5hi
KNO_3 (0.3%)	29 gh	4.95defg	32.5bcde	4.82efgh	30.5fg	4.5hi
KNO_3 (0.5%)	25 ij	6.125a	29gh	5.25bcde	24j	5.5b
Volk oil (1%) + KNO_3 (0.3%)	31.5 def	5.1cdef	32.5bcde	5.05def	31.75cdef	4.35t
Volk oil (1%) + KNO_3 (0.5%)	31ef	5.05def	31ef	4.9defgh	34.25b	4.27i

Means followed by different letters in each column indicate significant difference at $p < 0.05$ (Dunkan test).

The highest ounces were obtained in the control treatment of 'Haj-Abdollahi' (37) and the lowest ounces were obtained in 0.5 % potassium nitrate in 'Haj-Abdollahi' and Hasan-Abbasi cultivars (25, 24), respectively (Table 5).

In the 0.5 % potassium nitrate treatment, the highest weight of 10 kernels (6.12 g) was observed in 'Fandoghi' cultivar, and the lowest weight was obtained in the control treatment of 'Haj-Abdollahi' cultivar (3.45 g) (Table 5).

The results showed that 'Hasan-Abbasi' had the highest cluster weight (92.03 g), and the cluster weight of 'Haj-Abdollahi' and 'Fandoghi' were 77.76 and 60.53 g, respectively (Table 6).

'Hasan-Abbasi' cultivar had the highest number of pistachios in a cluster (47.82), and also 'Fandoghi' cultivar had the least number of fruits in a cluster (32.53) (Table 6).

The results showed that the 'Fandoghi' had the highest split percentage of the cluster (54.96%) and there was no significant difference between 'Hasan-Abbasi' and 'Haj-Abdollahi' cultivars (Table 6). Among the treatments, the control with 25.66% split percentage had the lowest split percentage, and there was no significant difference between the other treatments (Table 7).

Among the treatments, the 2% volk oil treatment and 1% volk oil treatment with 0.5% potassium nitrate had the lowest blank percentage of cluster (17.66%-17.16%, respectively), and the control treatment had the highest blank percentage of clusters (29.16%) (Table 7).

The use of 2% volk oil had the most positive effect on open-shell nuts, and the control treatments had the lowest percentage of open-shell nuts (Table 3), which is in line with the results of Kashanizadeh (2006) that the effect of foliar spraying of volk oil on commercial cultivars of pistachios was similar. They stated 6% volk oil increased the weight of nuts in cv. 'Qazvini' and other characteristics such as blank nuts and the dry weight of the whole fruit were also affected.

Table 6. Effect of cultivars type on pistachio cluster characteristics.

Cluster characteristics	Fandoghi	Hasan-Abbasi	Haj-Abdollahi
Cluster weight (g)	60.53c	92.03a	77.36b
Nuts/cluster	32.53c	47.82a	39.28b
Split percentage of cluster (%)	54.96a	51.21b	55.14b
Blank percentage of cluster (%)	21.32a	21.64a	21.42a

Means followed by different letters in each column indicate significant difference at $p < 0.05$ (Dunkan test).

Table 7. Effect of treatments on pistachio cluster characteristics.

Treatments	Open shell (%)	Blank (%)
Control	25.66e	29.16a
Volk oil (1%)	43.08d	25.83b
Volk oil (2%)	63.83ab	17.16e
KNO_3 (0.3%)	54.5c	22.5c
KNO_3 (0.5%)	62.58ab	20.41d
Volk oil (1%) + KNO_3 (0.3%)	60.16b	176.6e
Volk oil (1%) + KNO_3 (0.5%)	66.58a	17.5e

Means followed by different letters in each column indicate significant difference at $p < 0.05$ (Dunkan test).

Rahemi and Asghari (2004) also reported similar results when investigating the effect of volk oil, hydrogen cyanamide, and potassium nitrate on the cultivar 'Ahmad-Aghaei.' They used cyanamide at three levels (3%, 1.5%, 0), volk oil (7%, 3.5%, 0), potassium nitrate (3%, 1.5%, 0), and a combination of cyanamide and volk oil. Volk oil and potassium nitrate were used 4-8 weeks before bud break in two phases (January 5 and February 4). The use of these materials increased the yield of open shell nuts and decreased the percentage of blank nuts.

In a study, 4% and 6% volk oil treatments had the greatest effect on increasing the percentage of open shelled nuts and reducing the percentage of blank nuts (Javanshah et al., 2018). The results of Mahmoudi Meimand et al. (2022) also confirm the results of this research, and the use of volk oil increased the fresh and dry weight of nuts, decreased their blank nuts, and improved vegetative characteristics.

In one research, using potassium improved nut quality (fruit weight and percentage of split nuts) in pistachio orchards (Mimoun et al., 2004). Also potassium fertilization improved nut quality of mature pistachio trees (Zeng et al., 2001).

CONCLUSION

The results of the treatments carried out on the cultivars of 'Fandoghi', 'Hasan-Abbasi' and 'Haj-Abdollahi' showed that 2% volk oil caused earlier flowering of trees in all cultivars, and also increased the percentage of open shell nuts in 'Fandoghi' and 'Hasan-Abbasi' cultivars, decreased the percentage of blank nuts and increased cluster weight in 'Fandoghi' cultivar. The combination treatments of 1% volk oil with 0.3 and 0.5% potassium nitrate increased the ounce in all cultivars, decreased the percentage of blank nuts in 'Hasan-Abbasi' and 'Haj-Abdollahi' cultivars, and increased the percentage of open shell nuts in 'Haj-Abdollahi' cultivar. Potassium nitrate (0.5 %) increased the fresh and dry weight of 100 nuts and the weight of 10 kernels and decreased the ounce in all cultivars. In all cultivars, the control treatment had the lowest fresh and dry weights of 100 nuts, percentage of open-shell nuts, and the highest percentage of blank nuts. Therefore, considering global warming and the lack of chilling requirements for pistachio orchards, the use of these chemical compounds is effective in increasing the quantity and quality of the product.

Conflict of interest

The authors declare that they have no conflict of interests.

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Postharvest losses, causes and mitigation in tomato transportation: a systematic review

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ABSTRACT

Purpose: The study aimed to provide an overview of tomato loss during road transportation with specific interest in the causes of the postharvest loss, postharvest loss mitigation measures, as well as research focus and trends over the past few decades. **Findings:** Transport conditions significantly affect tomato quality, influenced by factors like vehicle specifications and road conditions, which contribute to mechanical damage. Post-harvest losses stem from various factors such as the usage of inadequate harvesting tools, inefficient handling and transport equipment, usage of inappropriate packaging materials, poor temperature management and rough handling of fresh fruits as well as substandard road infrastructure. These issues collectively result in substantial losses, reaching up to 60%, notably impacting developing countries. **Limitations:** The study focused on existing literature published in English. Consequently, it may not offer a comprehensive overview, as other studies with abundant information on the subject might be written in languages not covered by this study's language restriction. **Directions for future research:** Future research should prioritize investigating the impact of mechanical stress, such as vibration and impact loads, experienced by fruits like fresh tomatoes during road transport and material handling. Additionally, there is a need to assess the effectiveness of different packaging materials in safeguarding transported tomatoes against mechanical stress.

INTRODUCTION

The human body requires some quantity of minerals and vitamins as well as antioxidants for its physiological and anatomical growth. These constituents are abundant in tomato fruits, which make them suitable for nutritional and medicinal purposes, and the reason why the demand for tomatoes has been increasing for the past few decades (Wakholi et al., 2015; Mibulo et al., 2020; Marcus, 2013). In line with the aforementioned, governments all over the world have focused on increasing tomato production by 50-70% from the end of the 19th century (Elijah, 2021; Sheahan & Barrett, 2017). Tomato growth and yield are favoured by high altitude, high light intensity and low relative humidity (Tilahun et al., 2017). The selective performance causes tomatoes to be produced in some locations thereby increasing the transport distance between the areas of production and the areas of processing/consumption. Nigeria for example, the major tomato-producing states in Nigeria, namely, Bauchi, Borno, Benue, Kano, Kaduna, Plateau, Jigawa and Kwara states (Ugonna et al., 2015); these states are located within the Northern part of the country resulting in transport distance of between a few hundreds of kilometres between Lagos State (The nation's commercial capital) and states in the Central regions of Nigeria (e.g. Benue and Plateau States) to over 1000 km between the same commercial capital (South-Western Nigeria) and Borno State (North-Eastern Nigeria). Such a considerable transport distance between the said locations especially with the ride quality of roads as well as the environmental temperature makes it more challenging for tomato postharvest qualities.

Almost recently, tomato production has been projected to increase by up to 50% of the current production figures by the year 2050 (Stratton et al., 2021). More interestingly, the United Nations has set a goal to reduce post-harvest losses of fruits and vegetables, generally by up to 50%, which has attracted extensive research on the causes and potential mitigation measures (Affognon et al., 2015). While both statements seem to ensure global tomato security, yet, the post-harvest losses remain high (Bani et al., 2006; Sheahan & Barrett, 2017; Sugino et al., 2022), leaving serious questions as to the effectiveness of the current tomato postharvest mitigation measures.

To highlight the postharvest loss in tomatoes from general causes, postharvest loss figures from The Food and Agricultural Organization (FAO) for ten (10) randomly selected countries were examined for a period of nine (9) years (2010-2018) (FAOSTAT, 2022). It revealed that, across the examined countries, a total postharvest loss for tomatoes increased from 296.24 thousand metric tons in 2011 to 606.26 thousand metric tons in 2018 (Table 1). Failure to reduce tomato postharvest losses to acceptable levels despite numerous researches with documented published findings has further attracted several questions that pertain to the suitability and applicability of the hitherto suggested mitigation measures. What is the current trend in researching tomato postharvest losses? What factors are responsible for post-harvest loss of tomatoes and what measures are effective to reduce losses? To provide answers to the questions rose above, this study was planned and conducted based on a systematic search.

Table 1. Tomato postharvest loss from selected countries in Africa.

	Postharvest loss estimates per year (Thousand metric tons)									
Country	2010	2011	2012	2013	2014	2015	2016	2017	2018	Average by country
Ethiopia	55.64	81.73	55.73	39.37	30.7	65.21	28.4	41.2	43.8	49.09
Ghana	318.52	320.5	321	340.2	366.77	368.78	368.8	368.9	381	350.05
Kenya	539.15	396.54	444.86	494	443.3	402.5	410	507.1	599.5	470.77
Malawi	112.61	120.61	40.5	265.1	526.1	523	483.7	450	583.2	344.98
Nigeria	1799.96	1491.3	2060.3	1925.1	4083.5	4229.3	3412.7	4100	3913.99	3001.8
Rwanda	135	122.17	115	116.1	118.6	120.3	118.8	97.4	93.1	115.16
Uganda	31	30	35	34.95	36.2	38.00	39.4	40.98	39.46	36.11
Tanzania	300	350	390	423.3	387.8	400.4	403.8	359.8	356.09	374.58
Zambia	26	27	28.5	27.1	26.13	25.8	25.9	25.8	25.87	26.46
Zimbabwe	25	22.5	23.5	23.5	24	24.8	25.49	26.04	26.55	24.6
Average by year	334.29	296.24	351.44	368.87	604.31	619.61	531.5	601.72	606.26	

(Source: FAOSTAT, 2022).

METHODOLOGY FOR ARTICLE SEARCH AND INCLUSION

The systematic search was performed on January 14, 2023, with the Google Scholar search engine using the following search terms \"Transport-related Loss in Tomatoes\", \"Tomato Mechanical Injuries based on Road Transport\" AND \" Road transport Vibration effects on Fresh Tomato Cargo\".

Literature mapping and inclusion were conducted following the documented PRISMA guidelines (Moher et al., 2009). Only documents that matched all or part of the search terms were included and 150 documents were returned based on the initial search. The search was further refined by the year of publication being narrowed down to 2001 and 2023; as the range of publication years chosen is sufficient to show the trend of interest among researchers over more than three decades (34 years); this yielded 105 documents (conference papers, published articles and reports, as well as theses and dissertations).

To avoid duplication of articles, conference papers were isolated during review; as presented conference papers can be published and both versions can be found on the same search engine under the same search term, textbooks were also excluded from the search results; hence 54 published articles were used in the review.

To simplify the analysis, the review was further conducted and reported in the following subtopics; i) overview of reviewed articles, (ii) Tomato post-harvest loss estimates iii) Transport-related causes of tomato post-harvest losses iv) Proposed mitigation measures and related success.

OVERVIEW OF REVIEWED ARTICLES

Distribution of reviewed articles by research focus

54 articles published between the years 2001 and 2023 were used in this review, and this is composed of review articles (20.37%) and original research articles (79.63%). Most of the research focused on examining the effects of the relationships between road transport vibration levels and post-harvest loss of tomatoes (12.96%), the influence of packaging and cushioning materials (11.11%) and the estimation of the transport-related post-harvest loss in tomatoes (9.26%) and the application of modelling techniques in the study of roadside tomato losses (9.26%) (Fig. 1).

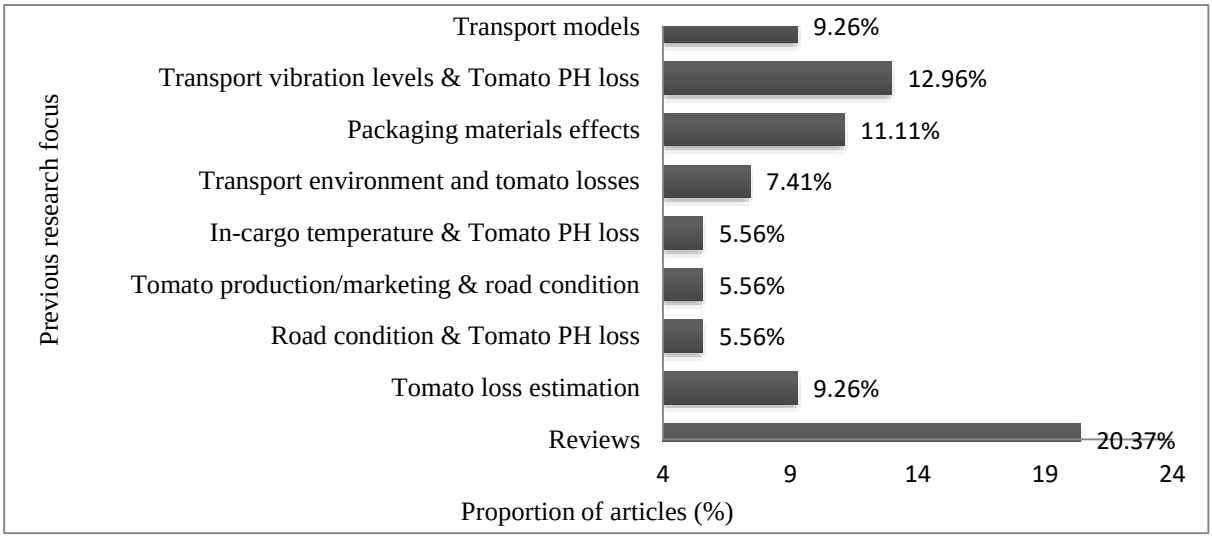

Fig. 1. Overview of the reviewed articles. PH: Postharvest.

Distribution of reviewed articles by continent

Contrary to most of the research fields conducted globally, which seem to indicate the USA and Europe spearheading most of the researches, this study revealed that most of the research on the postharvest loss of tomatoes were conducted in Africa and Asia. Of the 54 published articles reviewed in this study, most of the studies were conducted in Africa (70.4%) and Asia (20.4%), with the European continent indicating less research on the subject (Fig. 2).

The distribution of published articles observed for the various continents in this study followed previously published post-harvest loss estimates, in which Africa and Asia were known to have the highest post-harvest loss figures in fresh fruit and vegetables, generally (Arah et al., 2016; Bwade et al., 2019). The concentration of research in developing countries observed in this study is a good omen if the research results to reduce post-harvest losses are successfully implemented. The countries with the highest number of published articles on this topic are Nigeria (18 articles), South Africa (8 articles), Ghana (4 articles) and India (3 articles).

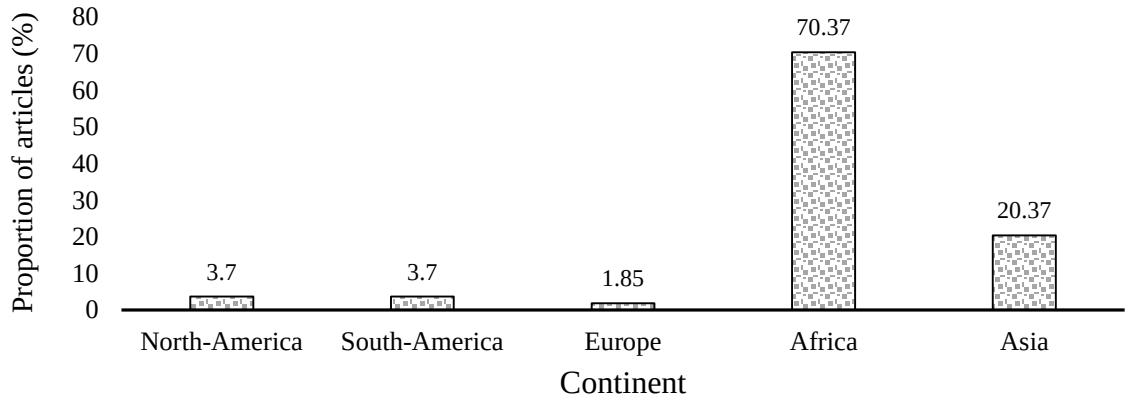

Fig. 2. Distribution of reviewed articles by continent.

Distribution of reviewed articles by year of publication

The articles used in this study covered the period between 2001 and March 2023, which were grouped into three decades (2001-2010, 2011-2020, and 2021-2023) to examine whether research interest in transport-related post-harvest losses in tomatoes is increasing or decreasing within the period under review (2001-2023); the stated range was chosen to provide an overview of the recent research trend on the reviewed subject matter (Fig. 3). The distribution of published articles per decade was highest in the second decade of the study (2011-2020) (59.3%). However, the average number of articles published per year showed a steady increase from 2.59 articles per year in the first decade of the study (2001-2010) to an average of 7.4 articles per year in 2023. This seems to indicate increased interest among researchers on the topic of research.

Transport-related postharvest loss estimates

Tomato is associated with considerably high moisture content at harvest (≤ 75%) (Bwade et al., 2019; Isack & Lyimo, 2015) and as with fresh horticultural materials, they continue to undergo a physiological metabolic process (Cherono & Workneh, 2018; Mutari & Debbie, 2011). During the post-harvest metabolic and physiological processes, nutrient reserves and moisture are depleted from the tissues of tomatoes; as a result, their quality variables (fruit weight, firmness, colour and chemical components such as vitamins, antioxidants and other minerals) change significantly (Cui et al., 2018).

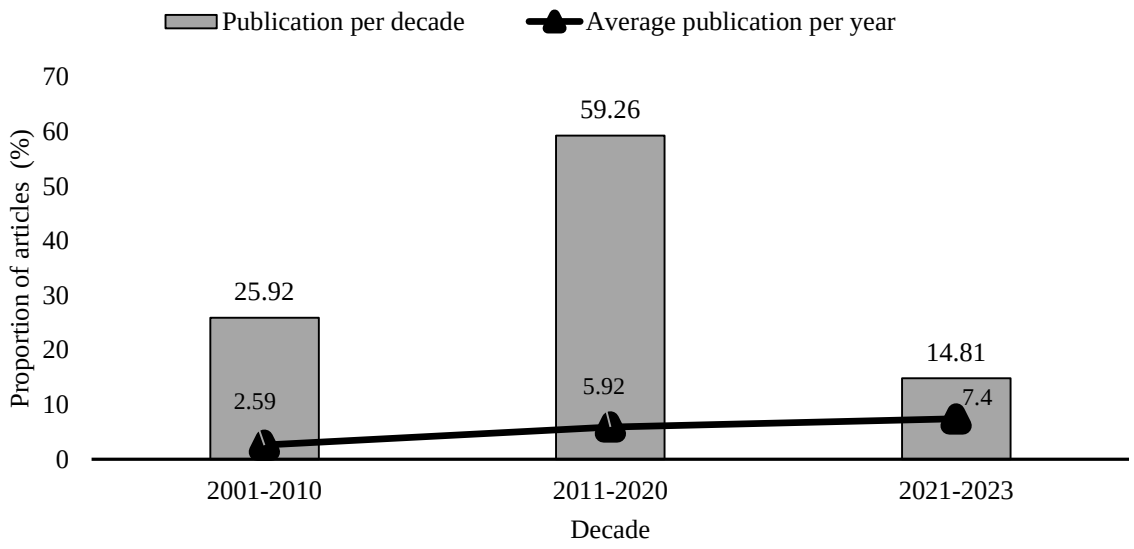

Fig. 3. Distribution of articles by year/decade of publication.

Regarding post-harvest losses of tomatoes and other food and agricultural materials, it is notable that countries and sub-regions with the greatest demands for food/agricultural materials have the highest percentage of post-harvest losses (Kamrath et al., 2016; Mibulo et al., 2020), maybe a cause for concern, but this shows the link between the technological/economic growth of the countries/sub-regions and the ability to implement post-harvest loss mitigation techniques. Several studies in the past have examined the post-harvest losses of tomatoes during transportation, some of the results are as follows: It has been reported that up to 40% of harvested tomatoes are lost to various spoilage agents in developing countries (Macheka et al., 2017). Higher tomato loss rates (up to 50%) have also been reported in sub-Saharan Africa (Mibulo et al., 2020). The post-harvest loss figures for fruit and vegetables are even much higher, as indicated by Sipho and Tilahun (2020) who studied the post-harvest loss of fresh fruit and vegetables in Africa by sub-region and reported the following post-harvest estimates in East Africa in Ethiopia (50%), Rwanda in Central Africa (30–80%), Ghana in West Africa (30–80%) and Swaziland in Southern Africa (20–50%).

Even related to the quantitative losses, researchers have relied on different methods for postharvest loss (PHL) estimation such as modelling, direct observation or residual methods, while other studies have relied on case studies that are not robustly representative of actual post-harvest loss statistics. The implication is that a PHL study performed on tomatoes within the same geographical area and cultivar wills most likely result in significantly different estimates of post-harvest loss, simply because different estimation methods were used. Second, the differences in the adopted PHL estimation method affect the validity of comparisons/conclusions drawn between independent PHL studies. From this, it can be concluded that there is a need to develop international protocols or guidelines that eliminate discrepancies between PHL estimation studies, which is very necessary since it will put an end to the conflicting figures published from PHL studies so far.

Postharvest loss causes and mitigation measures

As mentioned earlier post-harvest losses in tomatoes and other fresh horticultural materials can be quantitative, qualitative or economic, the severity of post-harvest losses depends on several factors (Idah et al., 2007b). The factors commonly associated with this are temperature and relative humidity management systems, packaging material, degree of ripeness at harvest, presence/absence of pre-chill treatment, road quality and vehicle type (Aba et al., 2012; Ileada & Ayodele, 2023). Other factors are insufficient or lack of efficient harvesting machinery/equipment and packaging/handling equipment. For example, in Nigeria, most of the tomato producers are small-scale farmers (60%), with medium and large-scale farmers accounting for 30 and 10% of tomato growers, respectively. Regardless of the category of farmers (small, medium or large farm), tomato harvesting and handling is done manually and in most cases with the help of poor or inefficient tools/packaging materials (Ugonna et al., 2015). The use of poor/inefficient harvesting equipment as well as packaging materials has been linked to significant mechanical damage to a variety of fresh fruits and vegetables such as tomatoes (Zaman, 2023; Waghmare et al., 2022).

The pursuit of a deeper understanding of the trend of causes of PHL in transported tomatoes, as well as the successes achieved in the implementation of proposed measures, has led to reviews with corresponding published results. Some of the reviews are as follows; an overview of packaging options for tomato smallholders in sub-Saharan Africa (Mibulo et al., 2020), factors responsible for tomato post-harvest losses and likely solutions (Bwade et al., 2019). Performance of multiple refrigeration systems (vacuum refrigeration, mechanical

refrigeration, hydro-refrigeration, and evaporative coolers) to identify the most appropriate systems for smallholder farmers (Sipho & Tilahun, 2020), factors influencing tomato quality losses (nutritional value and antioxidants), and income of farmers (Tilahun et al., 2017) and overview of post-harvest losses in tomato production in Africa (causes and possible solutions) (Arah et al., 2015). Transport-related factors are reviewed below.

Transport vehicle

In developing countries, tomatoes have been transported without refrigeration units on various modes of transport, including bicycles, motorcycles, donkeys, horses, cars, and trucks, resulting in significant damage to the produce (Bwade et al. 2019; Caixeta-Filho, 1999). Even with appropriate truck selection, significant tomato damage can still occur during transport due to factors such as the degree of ripeness of the crop, suspension system on the truck, payload capacity, road conditions, and driving speed (Cherono et al., 2018; Elijah, 2021; Lu et al., 2010b; Garcia-Romeu-Martinez et al., 2007; Jarimopas et al., 2005; Rissi et al., 2008; Zhou et al., 2015; Kefas et al., 2024). A review of the literature indicates that approximately 12.96% of the studies focused on the effects of transport vibration levels on post-harvest losses resulting from the road transport environment (Fig. 1).

Trucks that transport cargo respond to bumps on the road by oscillating along three perpendicular axes, i.e., vertical, longitudinal, and transverse. The impact load transferred to the truck body and its cargo depends on the intensity of vibration, which can cause damage to the product. To minimize the transmitted load and prevent cargo damage, a suspension system is required. Leaf springs, constant rate/coil springs, rubber springs, and air spring systems are the most commonly used suspension systems on trucks (Jarimopas et al., 2005; Ranathunga et al., 2010; Sittipod et al., 2009). Studies conducted in different countries, including China, Japan, South Africa, Spain, and Thailand, have verified the effectiveness of these suspension systems in absorbing shock/impact on agricultural cargoes, with air spring systems providing the best results compared to leaf spring suspension systems (Garcia-Romeu-Martinez et al., 2008; Ishikawa et al., 2009; Pretorius & Steyn, 2016; Zhou et al., 2015).

Although medium to high-speed transport is beneficial in delivering healthy tomatoes to consumers and processors, poor road conditions or unsuitable suspension systems can increase vibration/shock intensities during transport, leading to higher losses (Al-Dairi et al., 2021; Firdous, 2021). Therefore, a balance between ground speed and vibration/shock intensity is crucial to minimize fruit damage during tomato transport. Previous studies have also investigated the effects of cargo hold temperatures during tomato transport. Sugino et al. (2022) studied the effects of transport temperatures (0, 5, 10, and 20 °C) on post-harvest quality loss of tomato variety Rinka 409 harvested in different ripening stages. Although their study showed that lower loading temperatures (0-5 °C) resulted in reduced mass loss and reddening of the tomatoes during transport, they did not examine the effects of mechanical stress such as shock/vibration. The presence of such stress during transport can increase the rate of deterioration of the transported horticultural cargo, producing different results.

The findings suggest that the transportation of tomatoes in developing countries without proper refrigeration units and suspension systems can lead to significant damage to the produce. Even with appropriate truck selection, factors such as the degree of ripeness of the crop, payload capacity, road conditions, and driving speed can cause damage to the cargo. Although suspension systems such as leaf springs, constant rate/coil springs, rubber springs, and air spring systems have been shown to effectively absorb shock/impact on agricultural cargo, a balance between ground speed and vibration/shock intensity is crucial to minimize fruit damage during transport. Additionally, while lower loading temperatures have been

shown to result in reduced mass loss and reddening of tomatoes during transport, the effects of mechanical stress such as shock/vibration were not examined in a study by Sugino et al. (2022), suggesting a need for further research in this area. Overall, the weakness of these findings is that they are primarily based on studies conducted in specific countries and may not be generalizable to all developing countries. Additionally, the literature review suggests that only a small percentage of studies focus on the effects of transport vibration levels on post-harvest losses, highlighting a need for more research in this area (Bwade et al., 2019; Cherono & Workneh, 2018; Elijah, 2021; Lu et al., 2010a; Garcia-Romeu-Martinez et al., 2008; Jarimopas et al., 2005; Rissi et al., 2008; Zhou et al., 2015).

Road condition

Tomatoes can be transported by road, rail, ship or plane, but road transport is the most common mode, particularly in developing countries (Elijah 2021; Machado et al., 2020). The condition of roads varies based on road type (paved/unpaved), road design, and maintenance quality (Bwade et al. 2019; Sipho & Tilahun, 2020). When vehicles are driven on unpaved, rougher, and poorly maintained roads at higher speeds, the undulating nature of the roads increases the potential for damage to transported cargo, including tomatoes (Padilla et al., 2018). Figure 1 shows that around 5.56% of the research articles used in this study focused on the impact of road conditions on post-harvest losses of transported tomatoes.

The damage caused to tomatoes from transmitted vibrations and shocks during transportation depends on factors such as the ride quality of the road, measured by the International Roughness Index (IRI) (Mibulo et al., 2020), and vehicle speed (Pretorius & Steyn 2012; Zhou et al., 2015). The IRI ranges between 0 and 16 mm/m, with a smooth surface having good ride quality and an impassable road (Pretorius & Steyn, 2012). Ranathunga et al. (2010) found that roads with an IRI between 5 and 10 mm/m caused nearly four times as much damage to fresh agricultural produce cargo as good roads (0.9-2.0 mm/m). Cherono and Workneh (2018) evaluated the influence of packaging materials and ride quality on roads with an IRI value of 2.5 m/km (2.5 mm/m) and found that tomatoes transported on the road with the best ride quality (70% of the road length with IRI 2.5 m/km) had up to 10% higher marketability than those transported on other roads. Rather than using IRI to interpret ride quality, some studies suggest using power spectral density (PSD) to specify the energy content of shocks/vibrations generated when a truck travels on a particular road. This method takes into account the vibration response characteristics of the truck and the unevenness of the road profile and even identifies the vibration frequency at which cargo damage is most likely to occur (resonance frequency) (Singh et al., 2006; Widhiantari et al., 2016).

Some previous studies have recommended considering the resonant frequency of the transportation environment based on truck-roadway interaction for selecting packaging materials (Aba et al., 2012; Pretorius & Steyn, 2012). This is important because transporting packaged agricultural cargo at a natural frequency within the vibration frequency of the transport vehicle results in a significant amplification of shock/vibration amplitude, which imparts higher impact loads to the transported cargo, leading to much higher crop damage.

The studies on the impact of transportation on tomatoes have some weaknesses. One of the weaknesses is that there is a limited number of studies that have focused on the impact of road conditions on post-harvest losses of transported tomatoes, as only around 5.56% of research articles used in one study were focused on this aspect (Elijah, 2021; Machado et al., 2020). Additionally, while some studies suggest using power spectral density (PSD) to specify the energy content of shocks/vibrations generated when a truck travels on a particular road, there is no consensus on the best method to evaluate ride quality (Singh et al., 2006;

Widhiantari et al., 2016). Furthermore, some previous studies have recommended considering the resonant frequency of the transportation environment based on truck-roadway interaction for selecting packaging materials, but this has not been widely adopted in practice (Aba et al., 2012; Pretorius & Steyn, 2012). While some of the recent studies (Bwade et al., 2023) on road transport vibration and its effects on transported agricultural materials have highlighted the suitability of using International test protocols (such as ASTM D4196) for the evaluation of the safety of fresh agricultural materials within the road transport environment, yet such findings have not been independently validated. Nevertheless, more research is needed to better understand the impact of transportation on post-harvest losses of tomatoes and to identify best practices for packaging and transportation to minimize damage.

Packaging materials

Packaging materials are essential in the containment, preservation, and facilitation of handling and transportation of unitized loads, as well as providing some level of atmospheric modification for the packed produce (Bwade et al., 2019; Cherono & Workneh, 2018; Venus et al., 2013). Developed countries make use of plastic crates in handling and transporting tomatoes due to its benefits, while developing countries such as Nigeria, India, Ghana, and Egypt package tomatoes in a wide range of materials, such as jute sacs, nylon bags, raffia or cane-woven baskets, and wooden boxes (Arah et al., 2015; Idah et al., 2007a), with plastic crates used only among postharvest researchers (Anriquez et al., 2021). Various designs, shapes, and capacities of traditional baskets are used for packaging tomatoes. For example, raffia canes can be woven to fabricate a basket with a depth of 45-55 cm, a diameter of 60 cm, and a carrying capacity of 50 kg of tomatoes (Babarinsa et al., 2018). Other materials used include conical baskets with dimensions of 55 cm × 34 cm × 34 cm (top diameter × bottom diameter × depth), rectangular baskets with dimensions of 50 cm × 40 cm x 20 cm (length × width × depth) (Abubakar & El-Okene, 2015), carton boxes (8 kg capacity) and plastic bulk bins (468 kg storage capacity) (Cherono & Workneh, 2018), and wooden and plastic crates (Dari, 2018).

Previous studies have compared the performance of various tomato packaging materials. Cherono and Workneh (2018) studied the effects of packing tomatoes in large plastic bins (dimensions: 2 m × 1 m × 0.4 m; capacity: 468 kg) and small plastic crates (dimensions: 0.5 m × 0.4 m × 0.3 m; capacity: 20 kg). Kamrath et al. (2016) investigated the effects of lining the interior surfaces of wooden crates with paper or cloth on tomato post-harvest losses. Dari et al. (2018) examined the contributions of plastic and wooden crates at two levels of tomato capacity, respectively (30 and 50 kg), as well as the effects of cushioning materials (jute, paper, and foam) on the quality of tomatoes transported over a distance of 377 km. Babarinsa et al. (2018) evaluated the differences in the performance of traditional wicker and plastic crates in protecting the quality of tomatoes transported over a distance of 998 km. Abubakar and El-Okene (2015) compared the relative performance of two raffia baskets with conical and rectangular shapes and tomato capacities of 40 and 25 kg, respectively, over a transport distance of 877 km. Sibomana et al. (2018) evaluated the effect of packaging materials and chemical treatments (anolyte water and chlorinated water) on the post-harvest quality of tomatoes. Finally, Pretorius and Steyn (2019) studied the damage to tomatoes during transportation at different ripening stages (ripe-green, break, and red-ripe) and over a distance of up to 1050 km at a speed of up to 80 km/h.

While the studies mentioned provide valuable insights into the effects of packaging materials and transportation conditions on the post-harvest quality of tomatoes, it is important to note that most of these studies were conducted in specific regions and under specific

conditions, and may not be representative of the global tomato supply chain. Additionally, some studies have evaluated only a limited number of packaging materials, or have focused on a narrow range of transportation conditions. For example, Babarinsa et al. (2018) only evaluated the performance of traditional wicker and plastic crates in protecting the quality of tomatoes transported over a distance of 998 km, which may not be representative of all transportation conditions. Similarly, the study by Kamrath et al. (2016) only investigated the effects of lining the interior surfaces of wooden crates with paper or cloth on tomato post-harvest losses, which may not apply to other packaging materials or transportation conditions. Furthermore, many of the studies mentioned have focused on the effects of packaging materials and transportation conditions on the physical quality of tomatoes, such as bruising, decay, and weight loss, rather than on the nutritional quality or flavour of the fruit. This is an important limitation, as the nutritional quality and flavour of tomatoes are key factors in determining consumer satisfaction and demand. Therefore, while the studies mentioned providing valuable insights into the post-harvest quality of tomatoes, further research is needed to evaluate the performance of a wider range of packaging materials and transportation conditions and to assess the effects of these factors on the nutritional quality and flavour of the fruit.

Temperature

The degree of hotness or coldness of tomatoes and their transport/storage environment has been found to have a significant influence on postharvest physiological processes and enzymatic and microbial activities (Cherono et al., 2018; Tilahun et al., 2017; Venus et al., 2013). Lower temperatures have been found to retard these processes, resulting in the extended shelf life of tomatoes (Cherono et al., 2018). During transportation, in-cargo tomatoes receive heat from various sources, including the sun, ground surface, and respiration (Mutari & Debbie, 2011; Sipho & Tilahun, 2020). Temperature control is particularly challenging for closed trucks without temperature management systems, especially over long distances (Idah et al., 2007b).

Various cooling systems, such as traditional ventilated storage, mechanical refrigeration, and evaporative cooling, are used for tomatoes during transit and storage, each with its pros and cons (Macheka et al. 2017; Mogaji & Fapetu, 2011; Sipho & Tilahun, 2020). While there is a significant amount of research on the impact of temperature on tomatoes during transport and storage, there are still limitations and challenges associated with the available cooling systems and pre-chilling techniques. For instance, traditional ventilated storage is limited by its lowest achievable temperature and the risk of scratching or damaging tomatoes, while mechanical refrigeration is costly and may not be suitable for adoption among subsistence farmers (Macheka et al., 2017). Additionally, the performance of evaporative coolers may be limited by prevailing weather conditions, such as high relative humidity, although active evaporative coolers with incorporated desiccating units may help overcome this limitation (Sipho & Tilahun, 2020); the associated cost of maintaining the active evaporative coolers is higher. Furthermore, the effectiveness of pre-chilling techniques, such as ice water cooling and vacuum cooling, may also be impacted by logistical challenges and delays, resulting in the loss of transported tomatoes (Cherono et al., 2019). These limitations and challenges highlight the need for continued research and innovation to develop more efficient and affordable cooling systems and pre-chilling techniques for tomatoes during transport and storage.

CONCLUSION

This study provided an overview of tomato postharvest loss resulting from road transport vibration with a specific interest in the causes and mitigation measures employed based on previously published literature. The study yielded the following conclusions: Mechanical injuries to fresh tomatoes during transportation are greatly influenced by the characteristics of the vehicle used, the quality of road infrastructure, and the effectiveness of packaging materials. Additionally, factors such as temperature and gas concentrations experienced by tomatoes during transport and storage environments affect the severity of postharvest loss. Various cooling systems, including traditional ventilated storage, mechanical refrigeration, and evaporative cooling, are employed during transit and storage, each with its advantages and disadvantages. Future research should focus on investigating the effects of mechanical stress levels during handling and transportation. By addressing these areas, researchers can develop appropriate transportation systems and policies to ensure the delivery of high-quality tomatoes to consumers and processors in developing countries.

Conflict of interest

The authors declare that they have no conflict of interests.

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Leaf temperature and peroxidase activity of bearing pistachio cultivars

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ABSTRACT

Purpose: Environmental stresses are a main disturbing factor influencing horticultural productivity around the world. It will affect all plants including resistant or non-resistant cultivars. So, it is important to find the better cultivars and to check the response to adverse environmental conditions. **Research method:** Thus, the present research was conducted to evaluate responses of different bearing status of pistachio cultivars including Kalleh-Ghochi (K), Ohadi (O) and Ahmad-Aghaii (A), grafted on Badami-Rize-Zarand as rootstock, for six consecutive years to soil salinity. **Findings:** ON-bearing trees of 'Ahmad-Aghaii' showed the highest yield, followed by 'Ohadi' and 'Kalleh-Ghochi'. In addition, the lowest leaf temperature was observed with this cultivar. Although the highest nitrogen, sodium, carbohydrate, peroxidase and leaf temperature was obtained in ON-bearing trees, however, the lowest potassium and total phenol content indicated in OFF-bearing status. It was found a negative correlation between leaf potassium content and ABI, between leaf peroxidase activity and ABI and between leaf temperature and ABI. On the other hand, leaf temperature increased as leaf sodium content increased. **Research limitations:** There was no limitation. **Originality/Value:** From data presented here, it is concluded that salinity and Na accumulation might be effective in changing the response of the pistachio cultivar under harsh environmental conditions which affects yield component and alternate bearing index.

INTRODUCTION

Pistacia vera L. in Anacardiaceae (Ferguson & Kallsen 2016) is a dioecious plant (Crane & Iwakiri, 1985). The world production of pistachio is about 1 MT (FAOSTAT, 2020). Ashraf et al. (2008) stated that salinity accounted for 20 percent yield loss around the world, after high-temperature stress as a major environmental factor (40%). Currently, approximately 1,125 million hectares of land are salt-affected (Hossain, 2019). Generally, the pistachios are grown in areas with higher temperatures during growing season, where usually heat stress is dominant. In addition to heat and water scarcity, salinity stress should be added to cultivated areas. Plant metabolism activities are strongly affected by temperature fluctuations (Chaitanya et al., 2001; Levitt, 1980).

As literature shows, there are various recent reports regarding different aspects of pistachio trees from Iranian researchers, as Iran is one of the main producers of this valuable nut in the world (Azarmi-Atajan & Sayyari-Zohan, 2022; Pakdaman et al., 2023; Azarmi-Atajan et al., 2023; Miri et al., 2023; Eskandari Torbaghan, 2023). The alternate bearing disorder in fruit trees has been studied extensively (Barnett & Mielke 1981; Khezri et al., 2020; Goldschmidt & Sadka, 2021; Khayyat et al., 2018; El-Mardi et al., 2005). Genetic characteristics, environment, yield, and carbohydrate storage and mobilization are contributing factors to this habit. Khezri et al. (2020) evaluated pistachio alternate bearing in detail. Reports showed that ON-bearer pistachio leaves have more IAA and CKs compared with OFF-bearer trees (Okay et al., 2011). Moreover, ABA level was higher in most ON-bearing years compared with OFF-bearing status (Crane, 1986). Global warming is increasing and creating concerns about producing high-value products worldwide. The pistachio is categorized as a tolerant fruit tree to different harsh environments; however, we suppose that there are differences among pistachio cultivars. It is assumed that bearing intensity of pistachio cultivars might be influenced by stress conditions. However, there is no study regarding the correlation between leaf enzyme activities (peroxidase) and physiological performances in Iranian pistachios. In addition, there is no assessment regarding correlation between leaf temperature and alternate bearing index in pistachio cultivars. Thus, this study aimed to evaluate alternate bearing index (ABI) in pistachio cultivars including Kalleh-Ghochi, Ohadi and Ahmad-Aghaii grafted on Badami-Rize-Zarand and its correlation to leaf temperature, and to determine enzyme activity in parallel to leaf nutrient content during OFF and ON bearing statuses.

MATERIALS AND METHODS

The experiment was conducted in a commercial plantation in Bajestan suburban (34.5221° N, 58.1722° E), Razavi Khorasan, Iran, on 20 years old pistachio trees grafted on Badami-Rize-Zarand as rootstock in 2015 (OFF), 2016 (ON), 2017 (OFF), 2018 (ON), 2019 (OFF) and 2020 (ON) growing seasons. The study was done on three cultivars (V), including Kalleh-Ghochi, Ohadi and Ahmad-Aghaii, as uniform trees (190 ± 20 cm height), planted at a spacing of 6m between rows and 3m on rows. The selected trees were treated according to conventional farm management, for example, pruning, thinning, irrigation (ECw=6.01 dsm^{-1} as moderate salinity; pH=8.01), fertilization, and manuring. The soil type (Table 1) was deep, loamy and plants were fertilized using composted manure (2Kg per tree), urea (0.3Kg per tree), ammonium phosphate (0.2Kg per tree) and potassium sulphate (0.3Kg per tree) at essential amount regarding soil analysis results.

ABI evaluated based on Hoblyn et al. (1936) and Wood (1989) using the following formula (1):

$$I= (1/n-1)\ (a2-a1/a2+a1) + (a3-a2/\ a3+a2) +\cdots\ (an-1-n/an-1+) \qquad (1)$$

Where a = yield in corresponding years and n = number of years. If I = 0, there is no alternate bearing; if I = 1.0, there is total alternate bearing.

The total leaf-soluble carbohydrates were determined on the fully expanded leaves in July, August and September, according to Irigoyen et al. (1992) and glucose (0–100 mg l^{-1}, from MERCK) was used as a standard. The absorption at 625 nm was determined by a spectrophotometer (SHIMADZU AA-670, Japan). The equation used for standard curve preparation was y = 424.65 x – 13.176 (R^2 = 0.92). Total phenols were evaluated on the fully expanded leaves in July, August and September, based on Folin–Ciocalteu method (Singleton & Rossi, 1965), and the absorbance was measured at 725 nm using a spectrophotometer. Leaf temperature was measured in July, August and September, by a compact infrared thermometer with a laser pointer (Extech Instruments, Model 42500, Mini IR Thermometer, USA), on the newest fully expanded leaves. To measure the surface temperature of the leaves, five leaves were selected in each tree and the compact was placed 5cm from the surface and shot the gun to flash. Values represent the results for an average of 10 typical days each month with a clear sky from 12:00 to 2:00 pm. Enzyme activities were evaluated in July, August and September, in which all experiments were performed at 4°C. The leaf blades (10 g) were homogenized based on the method by Chaitanya et al. (2002). Peroxidase (POD, EC 1.11.1.7) activities were determined by the Putter (1974) using guaiacol at 436 nm. To reach this, 0.1 ml of the enzyme extract was added to the reaction mixture containing 0.05 ml guaiacol solution and 0.03 ml hydrogen peroxide solution in 3 ml phosphate buffer solution (pH 7.0). Then, the solution was mixed and the absorbance read at 436 nm using a spectrophotometer. Time was then noted for the absorbance to increase by 0.1. The enzyme activity was calculated using the extinction coefficient of guaiacol dehydrogenation product under the conditions specified. Chemical analysis (Chapman & Pratt, 1982) was carried out with oven-dried samples of leaves and inflorescence tissues in late July, which were ground separately and ashed at 550°C for 90 min in a porcelain crucible. The ash was re-suspended in hot 2 M HCl, filtered, made up to 50 ml with distilled water, and then used for K^+ and Na^+ analysis with a flame photometer (CORNING 405, Cambridge, UK). Nitrogen content was evaluated with Kjeldahl method (Zeraatgar et al., 2019). Fruit was harvested at the maturity stage in October, and the yield was assessed after hulling and drying, using a digital balance with an accuracy of 0.001g under consecutive bearing (B) statuses (OFF vs. ON).

Experimental design and data analysis

The experiment was conducted as factorial based on a complete randomized block design, with different cultivars (Kalleh-Ghochi, Ohadi and Ahmad-Aghaii) and bearing status (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON) as main and alternative factors, respectively. Three replications with six trees in each were used (18 trees for each cultivar). Statistical analysis of data was performed using ANOVA to determine statistically different values at significance levels of 0.05 and 0.01 based on LSD. All statistical analyses were performed using SAS version 9.2. The figures developed in Excel software.

RESULTS

Soil analysis showed high salinity (Table 1) and SAR value (29.65) for studied site. Soil status was not suitable for the mentioned cultivars. Leaf temperature showed significant changes with bearer and non-bearer trees, and ON-bearings showed the highest level of this variable. 'Ahmad-Aghaii' showed the lowest leaf temperature compared with other cultivars; however, the highest level of this variable was obtained with 'Ohadi' and 'Kalleh-Ghochi', during ON status (Table 2). Data showed a linear correlation between ABI and leaf temperature and it was clear that any increase in leaf temperature increases this index (R^2=0.61). Peroxidase activity (PXR) was evaluated for four consecutive years and data indicated higher values in ON-bearing trees. Moreover, 'Ahmad-Aghaii' showed the highest levels of activity compared with other cultivars. Regarding to sampling time, September showed the highest amounts of activity (data not shown). Interactive evaluation of Bearing× Cultivar (B×V) indicated the highest PXR in 'Ahmad-Aghaii' during ON-bearing status, compared with others (Table 2). A negative correlation was observed between ABI and PXR rate (R^2=-0.78). The highest total phenols were observed with OFF-bearing status, however, Ahmad-Aghaii cultivar showed the highest level of this variable in both bearing status (Table 2). Sampling times indicated that September with higher phenol contents compared with other times (July and August) (data not shown). Moreover, an exponential correlation was observed between ABI and total phenols (R^2=0.90), in which increasing leaf phenol contents raises the ABI.

Table 1. Chemical and physical characteristics of experimental soil.

ECe (dS m^{-1})	pH	Total N(%)	P	K^+	Zn^{2+}	Cu^{2+}
			(mg kg^{-1})			
6.05±0.2	8.49±0.10	0.36±0.03	5.37±0.3	153.04±0.21	1.91±0.50	1.63±0.41
Mn^{2+}	Fe^{2+}	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-
(mg kg^{-1})		(meq L^{-1})				
2.14±0.57	2.58±0.37	97.85±0.59	12.67±0.32	9.15±0.34	3.04±0.61	0.63±0.33

Data presented as mean± SD. The soil texture was sandy-loam. P (phosphorus), K (potassium), Zn (zinc), Cu (copper), Mn (manganese), Fe (Iron), Na (sodium), Mg (magnesium), Ca (calcium), Cl (chlorine) and HCO_3^- (bicarbonate).

Table 2. Interactive effects of bearing status and cultivar on total phenols and total carbohydrates within pistachio leaves tissue during consecutive years (2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

Bearing	Cultivar	Total phenols	Total carbohydrate	PXR	Leaf Temp.
		mg g^{-1} D.W.	mg g^{-1} D.W.	(Units mg $^{-1}$ chl min^{-1})	(°C)
	Ohadi	11.37	163.86	214.90	41.67
OFF	Ahmad-Aghaii	23.38	189.83	478.16	39.46
	Kalleh-Ghochi	16.32	81.86	308.72	42.86
	Ohadi	9.70	208.83	294.77	47.30
ON	Ahmad-Aghaii	11.78	211.49	572.42	41.91
	Kalleh-Ghochi	9.70	148.89	396.47	46.83
	Ohadi	14.55	161.67	218.24	39.72
OFF	Ahmad-Aghaii	25.81	169.68	490.57	41.02
	Kalleh-Ghochi	21.03	91.41	331.69	41.14
	Ohadi	11.51	219.93	306.22	51.56
ON	Ahmad-Aghaii	16.51	218.82	552.69	43.40
	Kalleh-Ghochi	16.71	148.08	394.40	45.13
LSD		1.541	7.258	9.538	1.816
Bearing status (B)		<.001	<.001	<.001	<.001
Cultivar (C)		<.001	<.001	<.001	<.001
B × C		<.001	<.001	<.001	<.001

ON: Bearing trees; OFF: Non-bearing trees. Total phenols assessed based on Galic acid; PXR: Peroxidase. Three replications each containing six trees were evaluated.

Total carbohydrate (TC) showed higher levels in leaves of ON-bearing trees, and the highest value was observed with 'Ahmad-Aghaii'. Higher TC is evidence of higher nut demand for this variable when the seed is grown. September sampling showed the highest level of this variable compared with others (July and August) (data not shown). Interaction between B×V showed the highest total carbohydrate in ON-bearer 'Ahmad-Aghaii', although there was no significant difference with 'Ohadi' (Table 2).

All studied cultivars showed alternate bearing and a remarkable yield fluctuation was observed. OFF-bearing 'Kalleh-Ghochi' showed a lower yield in the first two years (2015 and 2017), compared with others. On the other hand, ON-bearer 'Ohadi' and 'Ahmad-Aghaii' showed higher yield components (years 2016, 2018, and 2020; Fig. 1). Alternate bearing index (ABI) was evaluated (Fig. 2) and 'Kalleh-Ghochi' was indicated as a high alternate bearer, compared with others, followed by 'Ohadi' and 'Ahmad-Aghaii'.

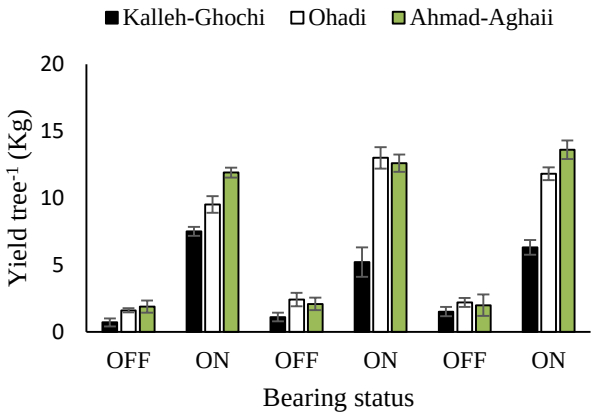

Fig. 1. Yield of different pistachios in ON and OFF bearing statuses from 2015 to 2020. Data represents means± SD. ON: Bearing trees; OFF: Non-bearing trees. Three replications each containing six trees were evaluated. (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

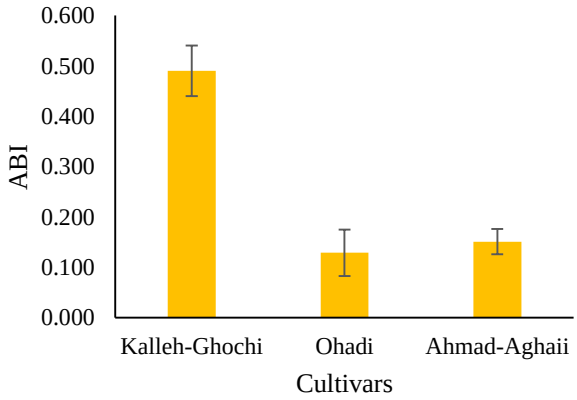

Fig. 2. Alternate bearing index **(**ABI) in different pistachio cultivars. Data represents means± SD. Three replications each containing six trees were evaluated. (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON)

Different leaf nitrogen contents were obtained based on bearing status (OFF vs. ON), and approximately higher levels of this variable were observed in ON bearer trees (data not shown). There was a significant difference in this variable when interactions were evaluated. In the second year of study (ON bearing status), all cultivars showed higher nitrogen content within leaf tissues (Table 3).

Regarding B×V, and in general, the highest potassium (K) content was observed within OFF-bearer leaf tissues followed by ON-bearers. Moreover, data showed that 'Kalleh-Ghochi' accumulated higher K levels within leaves compared with other cultivars (Table 3).

Evaluation of correlation showed a negative correlation between ABI and K contents within leaves (R^2=-0.60, data not shown).

Table 3. Interactive effects of bearing status and cultivar on nutrient concentration within pistachio leaves tissue during consecutive years (2015: 0FF; 2016: ON; 2017: OFF; 2018: ON; 2019: OFF; 2020: ON).

Bearing	Cultivar	Nitrogen	Potassium	Sodium	K/N	K/Na
		mg g^{-1} D.W.				
OFF	Ohadi	2.260	8.940	66.360	3.975	0.1355
	Ahmad-Aghaii	2.120	10.700	16.780	5.035	0.6686
	Kalleh-Ghochi	2.280	9.088	40.000	4.015	0.2289
ON	Ohadi	3.040	5.460	84.340	1.811	0.0664
	Ahmad-Aghaii	3.220	6.740	20.280	2.087	0.3334
	Kalleh-Ghochi	3.300	5.920	50.100	1.796	0.1186
OFF	Ohadi	2.240	8.270	62.800	3.694	0.1319
	Ahmad-Aghaii	2.200	10.840	17.400	4.937	0.6258
	Kalleh-Ghochi	2.360	8.730	37.240	3.772	0.2344
ON	Ohadi	2.320	4.546	87.040	1.963	0.0527
	Ahmad-Aghaii	2.320	6.700	22.300	2.889	0.3022
	Kalleh-Ghochi	2.240	5.760	54.020	2.577	0.1067
OFF	Ohadi	2.320	8.782	69.540	3.791	0.1265
	Ahmad-Aghaii	2.300	10.000	21.160	4.361	0.4799
	Kalleh-Ghochi	2.280	8.470	46.080	3.727	0.1844
ON	Ohadi	2.340	4.766	74.220	2.049	0.0646
	Ahmad-Aghaii	2.200	7.600	18.260	3.465	0.4279
	Kalleh-Ghochi	2.360	5.850	46.040	2.503	0.1284
LSD		0.2211	0.7352	6.0720	0.4138	0.0702
Bearing status (B)		<.001	<.001	<.001	<.001	<.001
Cultivar (C)		0.235	<.001	<.001	<.001	<.001
B × C		0.038	0.013	<.001	0.003	<.001

ON: Bearing trees; OFF: Non-bearing trees. Three replications each containing six trees were evaluated.

Regarding to sodium (Na) content, the simple effects of bearing, cultivar and interaction of B×V were significantly different. Results showed the highest Na levels in ON-bearing trees, specifically with 'Ohadi' and 'Kalleh-Ghochi' (Table 3). 'Ahmad-Aghaii' showed the lowest sodium accumulation within leaves. There was a positive correlation between ABI and Na accumulation within leaves (R^2=0.84, data not shown). Moreover, a positive correlation was observed between sodium accumulation in the leaf and leaf temperature (R^2=0.92, data not shown).

The potassium to nitrogen (K/ N ratio) was the highest in OFF-bearing status, compared with ON status, which might be resulted from lower N content in bearing trees. Moreover, 'Ahmad-Aghaii' showed a high level for this ratio compared with others (Table 3). There was an exponential correlation between ABI and K/N ratio (R^2=0.54).

The K to Na ration showed the highest values for the OFF-bearer Ahmad-Aghaii cultivar, compared with others. ON-bearer trees showed a lower value of this ratio (Table 3). The evaluation indicated a negative correlation between ABI and K/Na ratio (R^2=-0.64).

DISCUSSION

Soil analysis cleared harsh environmental conditions for the studied site, and also for mentioned cultivars, although, all of them were grafted on 'Badami-Rize-Zarand' as a resistant pistachio rootstock to drought and salinity (Adish et al., 2010; Rahneshan et al., 2018). There was a difference between leaf temperature of bearer and non-bearer statuses, with the highest values in ON-bearings trees. Comparing cultivars, 'Ahmad-Aghaii' showed the lowest value, and the highest level obtained with 'Ohadi' and 'Kalleh-Ghochi'. Higher leaf temperature influences photosynthetic apparatus and chlorophyll content, normal cellular homeostasis, electron transport, function of PSII because of enzyme degradation and inhibit Rubisco activase (Rca), carbohydrate assimilation and growth and development, glycolate pathway and caused H_2O_2 production, malondi-aldehyde (MDA) production due to lipid peroxidation, photo-inhibition, protein denaturation, enzymes and nucleic acid denaturation and accumulation of compatible solutes (Sade et al., 2011; Hasanuzzaman et al., 2013; Song et al., 2014; Bi et al., 2016; Moore et al., 2021). The oxidative stress further leads to cellular injury, including membrane protein breakage (Sade et al., 2011). An increase in leaf temperature will result in increases in VPD in natural environment, which affects photosynthetic induction by itself (Kaiser et al., 2017). It is suggested that 'Ahmad-Aghaii' exhibits different mechanisms to face high temperatures, and maintain PSII activity under harsh environments. ABI showed a positive linear correlation with leaf temperature (R^2=0.61), which may be because of running a lower rate of assimilation processes. Higher PXR activity was observed in ON-bearing trees and 'Ahmad-Aghaii' showed the highest levels of activity compared with two others. A negative correlation observed between ABI and PXR (R^2=-0.78), and higher PXR activity improved leaf photosynthetic assimilation which increases carbohydrate resources. We suggest that lower leaf temperatures might be resulted from higher PXR activity that was disagreement with results by Chaitanya et al. (2002), and Gulen and Eris (2004). More specifically, there is a correlation between PRX and catalase (CAT) enzyme activity and the appearance of physiological injuries caused by thermal stress, and its activity was enhanced by high-temperature stress (Kumar et al., 2012; Chalanika et al., 2017). In a study under heat stress, Gulen and Eris (2004) found an increase in PXR and decreased total protein content of strawberry plants. Peroxidase triggers the conversion of H_2O_2 to water and oxygen as an enzymatic defense of plant cells (Gaspar et al., 1982). Moreover, many other performances are related to PXR, including removing H_2O_2 and other toxic reductants, lignin production in cell walls, auxin catabolism, defensive responses

to wounding, defense against pathogen or insect attack, and some respiratory processes (Gaspar et al., 1982). CAT and PXR are chloroplast or cytosolic enzymes, which scavenge H_2O_2 generated primarily through SOD action. Hydrogen peroxide stimulates several different genes related to abiotic and biotic stress tolerances (Prasad et al., 1994). Under high temperatures, the reduction of H_2O_2 by ascorbate- glutathoine cycle is useful for dissipating energy and aids in adjusting ATP: NADPH ratios at times. Totally, Liu and Huang (2000) stated that any decrease in antioxidant enzyme activities under heat stress could contribute to damage of cell membranes.

OFF-bearer trees showed the highest total phenols, however, Ahmad-Aghaii cultivar showed the highest level of this variable in both bearing statuses. Certain PXR utilize the phenolic compounds and H_2O_2 to start the biosynthesis of several secondary metabolites that are essential for the plant growth, development and differentiation (Gaspar et al., 1991). Khayyat et al. (2018) stated that total phenols increase in vegetative organs of ON-bearing seedless barberry. It is suggested that bearer trees supply fruits with different substances to maintain sufficient yield, thus, lower phenols are not far away. Sampling times indicated the September with the higher phenol contents compared with other times (July and August) (data not shown) that was disagreement with Khayyat et al. (2018) on seedless barberry. An exponential correlation observed between ABI and total phenols (R^2=0.90).

Total carbohydrate (TC) showed higher levels in leaves of ON-bearing trees that was in agreement with Khayyat et al. (2018) but disagreed with Vemmos (2010), and the highest value observed with 'Ahmad-Aghaii'. Higher TC is evidence of higher nut demand for this variable when the seed is grown. At high temperatures, photosynthetic decline because of increased photorespiration and mitochondrial respiration, inactivation of Rubisco, decreased activity of photosystem II (PSII), and damage to the thylakoid membrane, which results in a reduction in ATP synthesis and increased thylakoid membrane permeability (Hozain et al., 2010). September sampling showed the highest level of this variable compared with others (July and August) (data not shown) that was disagreement with Khayyat et al. (2018). Research indicated that high temperatures also cause a rapid consumption of carbohydrates for maintenance (Teskey et al., 2015). The mentioned reasons might be the leading causes of lower TC with other cultivars in our experiment. It is thought that respiration might be the main cause of decrease in the TC reservoirs within leaves. Moreover, lower PXR levels in mentioned cultivars might be the cause of higher TC consumption under harsh environments.

All studied cultivars showed alternate bearing and a remarkable fluctuation of yield was observed. The yield components of trees might be resulted from genetic background and environmental stresses influence on tree performance (Goldschmidt & Sadka, 2021). In our study, similarity on soil conditions and rootstock evidence that genetic background might be a main cause of any differences among cultivars. It is believed that plants whose fruit ripens early in the growing season or plants having a longer PRFP (post-ripening foliation period) and vegetative growth will accumulate a larger pool of assimilates and thus show lower ABI compared with those that ripen late in the season (Khayyat et al., 2018).

Higher levels of leaf nitrogen observed in ON bearer trees (data not shown) that was in disagreement with Elmardi et al. (2005) and in agreement with Brown et al. (1995) findings. Brown et al. (1995) found a huge depletion of nitrogen accumulation in OFF bearer pistachios, after ON status.

The highest potassium (K) content observed in OFF-bearing trees followed by ON status, that was disagreement with Brown et al. (1995). Elmardi et al. (2005) stated that more potassium and sodium observed within ON-tree leaves compared with OFF status. We suppose that higher K accumulation in OFF bearing leaves might be the prerequisite for its translocation and accumulation in perennial plant part for next season (ON status). Evaluation

of correlation showed an exponential correlation between ABI and K contents within leaves (R^2=0.60). Regarding to potassium content, it is assumed that leaf potassium sufficiency led to lower leaf temperature, may be because of higher RWC, specifically under hot environments, which could be a suitable characteristic for screening heat-tolerant cultivars.

Results showed the highest Na levels in ON-bearing trees that were in agreement with Elmardi et al. (2005). There was a significant difference among cultivars and 'Ahmad-Aghaii' showed the lowest sodium accumulation within leaves. There was a negative correlation between ABI and Na accumulation within leaves (R^2=-0.84, data not shown). Moreover, a positive correlation observed between sodium accumulation in leaf and leaf temperature (R^2=0.92, data not shown). Increased leaf temperature might be related to lower RWC or lower content of osmolites within photosynthetic tissues. Thus, lower entrance of Na^+ could be applied as a good character for breeding or selection.

The potassium to nitrogen (K/ N ratio) was the highest in OFF-bearing status, compared with ON status, which might be resulted from lower N content in bearing trees. There was an exponential correlation between ABI and K/N ratio (R^2=0.54). The K to Na ration showed the highest values of OFF-bearing status of Ahmad-Aghaii cultivar, compared with others. Evaluation indicated an exponential correlation between ABI and K/Na ratio (R^2=0.64). Potassium ions are highly involved in the activation of several enzymes essential for cellular functions (Tester and Davenport 2003). When plants are exposed to sodium chloride (NaCl) induced salinity, the influx of Na^+ and Cl^- impairs the transport of other ions, such as K^+ and Ca^{2+} (Binzel et al., 1988). In such conditions, protein synthesis and enzyme activation processes are directly affected by the ability of plants to select K^+ at the expense of Na^+ ions (Kamiab et al., 2012). We supposed that bearer trees are highly demanding for potassium accumulation in fruits that decrease the K/ Na ratio within leaves.

CONCLUSION

Data showed the ON-bearing 'Ahmad-Aghaii' with the highest yield, followed by 'Ohadi' and 'Kalleh-Ghochi'. The lowest leaf temperature observed with this cultivar. ON trees showed the highest nitrogen, sodium, carbohydrate, peroxidase and leaf temperature, however, the lowest potassium and total phenol content observed with OFF-bearers. ABI showed an exponential correlation with potassium and peroxidase activity. It is concluded that Na accumulation within leaves influence the growth response of pistachio trees under harsh environmental conditions, might be related to enzyme activity and leaf temperatures, and lead to change in yield and alternate bearing index.

Competing interests and declarations

All authors declare that they have no competing financial interests and non-financial interests related to this work and its publication. We emphasis that we did not receive any grants from any organization.

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Sulphur dioxide sheet and cold storage synergy for post-harvest management of Thompson seedless grapes (*Vitis vinifera*)

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ABSTRACT

Purpose: This study examined the influence of various storage conditions on Thompson seedless (*Vitis vinifera*) grapes quality. **Research method:** Grapes were stored under four conditions: control (room temperature i.e., 20-22°C, no SO_2), T1 with SO_2 sheets at room temperature i.e., 20-22°C, T2 with SO_2 sheets in cold storage at 1°C and T3 without SO_2 sheets in cold storage at 1°C. Changes in acidity, total soluble solids (TSS), total anthocyanin content, total phenols, sugars (glucose and fructose), trans-resveratrol, decay %, weight loss % and antioxidant activity were monitored over 60 days. **Findings:** The findings revealed a synergistic effect between SO_2 and cold storage. Grapes stored with both SO_2 sheets and cold storage (T1) exhibited the slowest decline in anthocyanin, phenols and antioxidant activity of 211.06 mg/L, 2102.39 mg/L and 7.19 mM DPPH, respectively after 60 days. T1 grapes found to have slower reduction in sugars and trans-resveratrol concentration i.e., 15.47 to 15.37 g/100mL and 695 to 516 µg/g, respectively compared to control samples 15.47 to 14.81 g/100mL and 695 to 500 µg/g, respectively. **Research limitations:** The study focused solely on storage conditions of Thompson seedless variety grapes, limiting the generalizability of the findings to different grape varieties and maturity levels. **Originality/value:** These results highlighted the importance of proper storage techniques, particularly the combined use of SO_2 and cold storage, for maintaining grape quality and extending shelf life.

INTRODUCTION

Fruit production is one major way that the agricultural industry contributes to the global economy (Mohamed et al., 2011). Grapes (*Vitis vinifera*) are among the most economically significant fruits. Post-harvest losses continue to be a significant obstacle for grape growers and supply chain operators across the globe (Zhou et al., 2017; Mirfatah et al., 2024). In addition to having an effect on financial gains, post-harvest losses waste resources increase food insecurity and environmental damage (Ali et al., 2021; Moradinezhad & Ranjbar, 2023). The goal of post-harvest management techniques is to reduce losses and preserve fruit quality from harvest to eating. When it comes to prolonging the shelf life of perishable fruits such as grapes, cold storage is one of the most useful strategies used (Chaves & Zaritzky, 2018). Fruit quality and freshness are preserved through the slowing down of physiological processes including respiration and ripening during cold storage (Brizzolara et al., 2020). Cold storage might not be enough to stop all degradation and deterioration, though, particularly in fruits like grapes that are prone to oxidative browning and fungal infections.

The food industry frequently uses sulphur dioxide (SO_2) as a preservative because of its antibacterial and antioxidant qualities. Because of its ability to stop enzymatic browning and fungal growth, it is a great option for improving grape quality after harvest (Palou et al., 2010). The SO_2 sheets contain sodium metabisulfite enclosed between paper sheets of differing permeability. When moisture within the package of grapes is absorbed by the pads, it reacts with the sulphite, releasing SO_2. The quick-release part of the pad gives a flush of SO_2, which peaks after about 24 hours and then diminishes in about a week (Lichter et al., 2008). In the past, SO_2 has been applied through fumigation or by using sachets or pads that release SO_2 inside storage containers. These techniques do have certain drawbacks, such as unequal SO_2 dispersion and possible health risks from prolonged exposure to sulphur dioxide. New developments in post-harvest technology have resulted in the creation of sheets that release sulphur dioxide and are intended for use in cold storage facilities. With the regulated release of SO_2 offered by these novel sheets, human exposure to the gas is reduced and equal dispersion inside the storage space is ensured. Sulphur dioxide sheets and cold storage work together to provide a synergistic strategy to grape post-harvest management that successfully addresses enzymatic deterioration as well as microbiological spoilage.

Although combining sulphur dioxide sheets with cold storage may have advantages, there was a lack of research on how well these two approaches work together to maintain grape quality during post-harvest handling. Previous studies have mainly looked at individual methods rather than how they work together as a synergistic whole. Therefore, more thorough research is required to determine how well this integrated approach works to prolong grape shelf life and preserve grape quality during storage and transportation. By assessing the complementary benefits of cold storage and sulphur dioxide sheets on grape post-harvest management, this research seeks to close this gap. The research aims to clarify the mechanisms behind the combined action of these techniques and their influence on important quality indicators such fruit firmness, colour retention, microbial load and sensory qualities of grapes under cold storage conditions.

MATERIALS AND METHODS

Thompson seedless grapes (*Vitis vinifera*) were procured from the vineyards in the Nashik, Maharashtra, India. According to AGMARK maturity requirements, grapes must have a minimum TSS of 16 °Brix and a sugar-to-acid ratio of 20:1 (Apeda, 2021). Grapage (grape

guard sheets/SO_2 pads) SO_2 sheets with maximum residue limit of 10 ppm sulphite and absorbent papers were purchased from JK Enterprise, Nashik, Maharashtra, India.

Grape bunches that met maturity requirements were chosen for vineyard harvesting. Before the temperature of the berries rose over 20°C, the grapes were harvested in the early morning. Expert harvesters with sharp scissors and soft rubber gloves had completed the task. Grapes were harvested and then taken to the packhouse one day before they were picked. There, broken berries and malformed, decaying, small, and discoloured berries were removed by cutting their pedicels off of the chosen bunches using long-nosed scissors, and grapes were graded. Grapes were graded and then placed in plastic clamshell punnets. To prevent the grapes from bruising, a layer of bubble pad and protective liner was positioned at the bottom of the box after the punnet was filled. General steps of packaging of harvested grapes are shown in Figure 1.

Grapes packed without sulphur dioxide sheet and stored at room temperature

The grape punnet was put inside a box, sealed with a liner, and fresco pad without sulphur dioxide sheet. Grapes were kept in dark at room temperature at 20-22°C after packaging (control). Grapes were taken to laboratory for the quality analysis at 10 days' interval up to 60 days. These samples were used as a control sample to compare with other treatments.

Grapes packed with sulphur dioxide sheet and stored at cold storage

The grape punnet was put inside a box, sealed with a liner, and covered with a sheet of sulphur dioxide and a fresco pad. Grapes were kept for pre-cooling after packaging in order to lower their temperature to less than 4°C in 6 to 8 hours. The purpose of pre-cooling is to lower field heat. Grapes were refrigerated in cold storage at 1°C and 90-95% RH in dark after being pre-cooled (T1). Grapes were taken to laboratory for the quality analysis at 10 days' interval up to 60 days.

Grapes packed with sulphur dioxide sheet and stored at room temperature

The grape punnet was put inside a box, sealed with a liner, and covered with a layer of sulphur dioxide and a fresco pad. Grapes were kept in dark at room temperature at 20-22°C after packaging (T2). Grapes were taken to laboratory for the quality analysis at 10 days' interval up to 60 days.

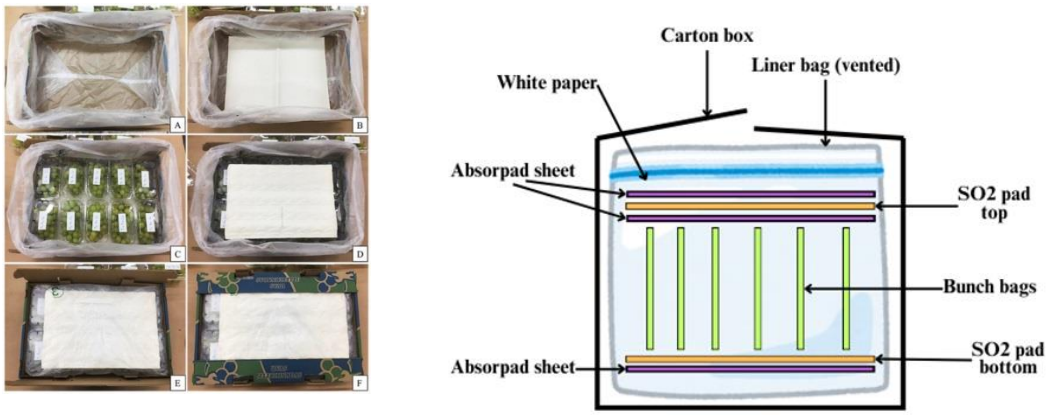

Fig. 1. Packaging steps and diagram for harvested grapes adopted and modified from de Aguiar et al. (2023).

Grapes packed without sulphur dioxide sheet and stored at cold storage

The grape punnet was placed within a box, lined, and fresco pad covered without sulphur dioxide sheer. After packaging, grapes were kept for pre-cooling in a cooling chamber for a minimum of 6 to 8 hours to bring their temperature down below 4°C. Pre-cooling is done to reduce field heat. Grapes were pre-cooled and then placed in cold storage at 1°C and 90-95% RH in dark (T3). At 10 days' interval up to 60 days, grapes were brought to laboratory for analysis.

Analysis of grapes quality parameters

Titratable acidity was measured using an automatic titrator (Mettler Toledo EasyTitration, Mumbai, India) where results were measured in % of malic acid. Total soluble solids (TSS) was measured using a hand-held refractometer (Erma, Tokyo, Japan) with results measured in °Brix. Total anthocyanin content was measured using UV-vis spectrophotometer (Jenway® 6305, Mumbai, India) at 520 and 700 nm expressed in mg/L, method described by Pastrana-Bonilla et al. (2017). Total phenols were analysed by Folin-Ciocalteu method described by Way et al. (2020) using UV-vis spectrophotometer at 765 nm expressed in mg/L. Sugars i.e., glucose and fructose presented in grapes were estimated by following the method described by Albalasmeh et al. (2013) using sulphuric acid and UV-vis spectra at 315 nm. The antioxidant capacity of the sample extract was measured as per the method suggested by Brand-Williams et al. (1995) and modified by Sanchez-Moreno et al. (1998). DPPH is one of the stable and commercially available organic nitrogen radicals and has UV-vis absorption maxima at 515 nm. On reduction of the colour solution fades and the reaction progress is monitored with a spectrophotometer at 515 nm. In methanolic solution (0.1 ml) of sample extract (15 mg/ml) added to 3.9 ml of DPPH (0.025g/ l) in methanol and absorbance measured at 515 nm. The absorbance was measured until the reaction reached a plateau (steady state). Estimation of trans-resveratrol was carried out using the method described by Camont et al. (2009) at 304 nm uv-viz absorption in uv-vis spectrophotometer. Decay % and weight loss % were calculated using the following formulas (1 and 2).

$$\text{Decay \%} \frac{\text{Decayed grapes (g)}}{\text{Initial weight (g)}} \times 100 \qquad (1)$$

$$\text{Weight loss \%} \frac{\text{Measured weight (g)}}{\text{Initial weight at the beginning of storage (g)}} \times 100 \qquad (2)$$

Statistical analysis

All the data were expressed as mean ± standard deviation (SD) of three determinations. Data obtained during the study was analysed and Completely Randomized Design (CRD) was performed using Design Expert 13 software via a one-way analysis of variance (ANOVA). At each 10-day interval (up to 60 days), randomly selected three number of punnets from each treatment were analysed for quality parameters. P values of less than 0.05 ($P < 0.05$) were considered as significant.

RESULTS AND DISCUSSION

Grapes were packed according to steps described by de Aguiar et al. (2023) as shown in Figure 2 with modification in conditions. Packed grapes with SO_2 sheets were stored in cold storage and room temperature. Grapes without SO_2 sheets were stored in cold storage. Control sample were stored at room temperature without SO_2 sheets. Physico-chemical analysis of grapes stored in different conditions for 0 days and 60 days are presented in Table 1.

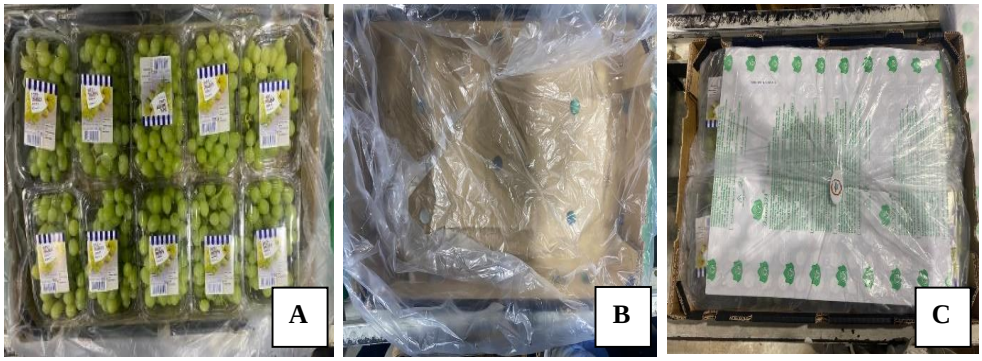

Fig. 2. Grapes packed in different conditions (A) control, (B) without SO_2 sheet, and (C) with SO_2 sheet.

Table 1. Physico-chemical parameters of grapes stored in different conditions.

Parameter	Acidity (% malic acid)		Sugar (° Brix)		Total anthocyanins (mg/L)		Total phenols (mg/L)		DPPH antioxidant activity (mM)	
Days	0	60	0	60	0	60	0	60	0	60
Control	0.92 ±0.01	0.78 ±0.01	17.50 ±0.11	14.56 ±0.15	212.33 ±0.15	190.00 ±0.13	2115.12 ±0.20	1856.25 ±0.11	7.22 ±0.04	5.76 ±0.01
(T1)	0.92 ±0.01	1.03 ±0.02	17.50 ±0.11	16.50 ±0.10	212.33 ±0.15	211.06 ±0.22	2115.12 ±0.20	2102.39 ±0.12	7.22 ±0.01	7.19 ±0.02
(T2)	0.92 ±0.01	1.12 ±0.02	17.50 ±0.11	14.80 ±0.12	212.33 ±0.15	205.25 ±0.10	2115.12 ±0.20	2085.74 ±0.20	7.22 ±0.01	7.10 ±0.01
(T3)	0.92 ±0.01	1.95 ±0.02	17.50 ±0.11	15.66 ±0.10	212.33 ±0.15	199.22 ±0.10	2115.12 ±0.20	1992.56 ±0.12	7.22 ±0.02	6.84 ±0.05

T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes.

Changes in acidity

Changes in acidity were measured as the percentage of malic acid shown in Figure 3. Control samples shown the decrease in acidity from 0.92% to 0.78%. Fresh grapes, not being subjected to cold storage, would have their natural metabolic processes ongoing. These processes include the conversion of malic acid to other forms of acid or to energy, led to a decrease in the percentage of malic acid over time. In T1 samples, the acidity slightly increased from 0.92% to 1.03%. The increase in acidity could be due to the cold storage slowing down the metabolic processes, including the conversion of malic acid to other forms (Yan et al., 2022; Deng et al., 2005). The use of SO_2 sheets helped in inhibiting microbial activity that could otherwise contribute to the breakdown of malic acid (Zhan et al., 2023; Chervin et al., 2012). In T2 samples, the acidity increased from 0.92% to 1.12%. The room temperature allowed more active metabolic processes, led to a higher conversion rate of other acids into malic acid. The SO_2 sheet, while inhibiting microbial activity, would not have been as effective in slowing down these processes as cold storage (Zhan et al., 2023; Lakso & Kliewer, 1975). In T3 samples, the acidity increased significantly ($p < 0.05$) from 0.92% to 1.95%. Without the SO_2 sheet, the grapes were more exposed to microbial activity which could lead to the production of more malic acid (Ahmed et al., 2018). However, the cold storage slows down these processes, which is why the increase in acidity is not as drastic as it could have been at room temperature.

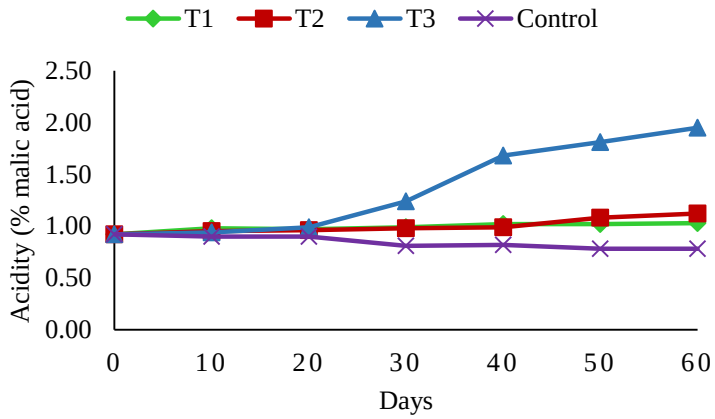

Fig. 3. Changes in acidity (% malic acid) of the grapes stored at different conditions.

(T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

Changes in total soluble solids and sugars

It was found that the sugar content (TSS) in grape juice decreased over time during storage, regardless of the storage conditions. This decrease was significant from 17.5 to 14.8 °Brix in grapes stored at room temperature with SO_2 sheets (T2) and in grapes stored in cold storage without SO_2 sheets (T3) i.e., 15.66 °Brix after 60 days. Fresh grapes also witnessed a decrease in sugar content over time as shown in Figure 4. SO_2 sheet combined with cold storage (T1) gave the highest preservation of TSS i.e., 16.5 °Brix after 60 days.

Grapes continue to respire after harvest like the other fruits and berries. During respiration, sugars broken down into carbon dioxide and water, which resulted a decrease in sugar content (Zhong et al., 2023). The use of SO_2 sheets found to slow down the decrease in sugar content. SO_2 is a common preservative used in winemaking and other food industries due to its antioxidant and antimicrobial properties. It can inhibit the activity of many enzymes, slowing down the metabolic activities in the grapes and thus resulted the decrease in sugar content. Cold storage also seems to slow down the decrease in sugar content by reducing the rates of respiration, fermentation and other metabolic activities (Vlassi et al., 2018). Similar results were observed by Ahmadi Soleimanie and Vafaee (2023), where they found that total soluble solids (TSS) content of Iranian grape cultivars slowly increased during cold storage up to day 21, particularly in the Sahebi cultivar. In another study by Leng et al. (2022) shown that grapes stored at low temperature, significantly reduced the decay incidence, weight loss, rachis browning.

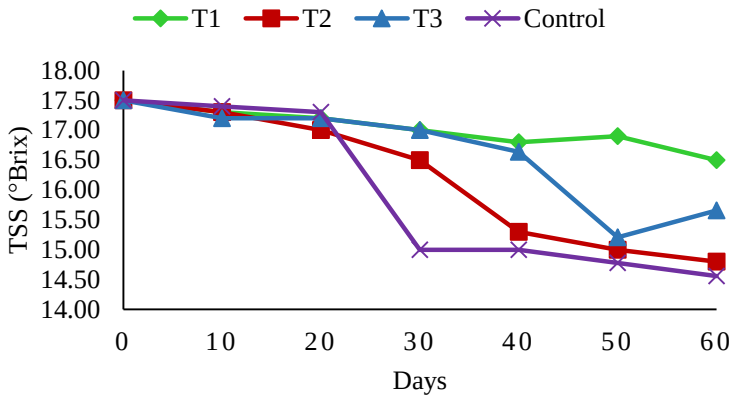

Fig. 4. Changes in TSS of grapes stored in different conditions.

(T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

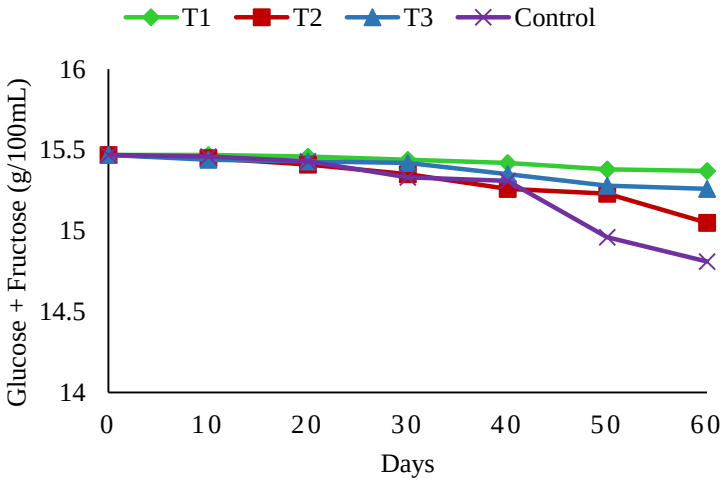

Fig. 5. Changes in grapes sugars (glucose and fructose) in different conditions.

(T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

In control samples, the sugar content decreased was significant ($p < 0.05$). Without the protective effects of SO_2 and cold storage, the metabolic processes of grapes and microbial activity occurred rapidly, which led to a rapid decrease in sugar content. The Brix level in control samples decreased quite significantly from 17.5 to 14.56 (Fig. 5). This indicated a gradual reduction in sugar content, which was a result of faster metabolic processes at room temperature without SO_2 protection. In T1, the sugar content remained relatively stable. This is likely due to the use of sulphur dioxide (SO_2), which is commonly used in winemaking and food preservation for its antimicrobial and antioxidant properties. Cold storage also found to be slowed down the metabolic processes, including sugar conversion. The Brix level in T1 decreased slightly from 17.5 to 16.5. This suggested a low level of reduction in sugar content, which related to the stable glucose and fructose levels observed. In T2, the sugar content

decreases significantly ($p < 0.05$). Here, the SO_2 sheet helped to preserve the grapes while the warm storage temperature accelerated the metabolic processes. This resulted faster conversion and consumption of sugars. The Brix level in T2 decreased significantly from 17.5 to 14.8. This indicated a high level of reduction in sugar content, resulted from the accelerated sugar consumption at room temperature. In T3, the sugar content decreases slightly. Without the protective effects of SO_2, the grapes were more susceptible to microbial activity and consumption of the sugars. However, the cold storage managed to slow down these processes. The Brix level in T3 fluctuated and ended up slightly lower than it started (17.5 to 15.66). This suggested some variability in sugar content, possibly due to the lack of SO_2 protection.

Changes in total anthocyanin

Total anthocyanin content in grapes under different storage conditions over a period of 60 days are presented in Figure 6. In control sample, the anthocyanins in fresh grapes degraded the most rapidly without any preservation methods. The lack of SO_2 allowed for enzymatic browning to occur. The anthocyanin content decreased significantly from 212.33 to 190 mg/L ($p < 0.05$) without cold storage to slow down these reactions in control sample. The anthocyanin content remained relatively stable in T1 with only minor decrease observed. This could be attributed to the protective effect of sulphur dioxide (SO_2) (Lichter et al., 2008). It worked by inhibiting the action of polyphenol oxidase, an enzyme that contributed to the browning of fruits and the degradation of anthocyanins (Ahmed et al., 2018). Cold storage further slowed down these enzymatic reactions and the growth of spoilage microorganisms (Elatafi et al., 2023). Therefore, the combination of SO_2 and cold storage found to be the most effective preservation of anthocyanins. The anthocyanin content decreased more significantly compared to cold storage. In T2, the SO_2 provided some protection against enzymatic browning but the higher storage temperature accelerated these reactions. Heat can provide the energy needed for chemical reactions, including those that degrade anthocyanins. Therefore, even with the use of SO_2, the anthocyanin content decreased more significantly up to 205.25 mg/L at room temperature (Elatafi et al., 2023; Muche et al., 2018). In the absence of SO_2, the protective effect against enzymatic browning was lost in T3. Even though cold storage can slow down these reactions, the lack of SO_2 led to a more significant decrease up to 199.22 mg/L ($p < 0.05$) in anthocyanin content. This stated the importance of SO_2 in the preservation of anthocyanins (Ahmed et al., 2018; Lichter et al., 2008).

Changes in total phenols

The phenols content in control samples significantly decreased over time, starting from 2115.1 mg/L and dropped to 1856.25 mg/L as shown in Figure 7. This was due to natural degradation processes. The phenols content remained relatively stable in T1, with a slight decreased from 2115.1 mg/L to 2102.39 mg/L. This suggested the use of SO_2 sheets and cold storage effectively preserve phenols. The use of SO_2 sheets seems to have a preserving effect on the phenols content, especially when combined with cold storage. Similar trend was observed by Antoniewicz et al. (2021) indicated that storage conditions and time affect the antioxidant activity and polyphenol content. The phenols content decreased more significantly in T2 i.e., from 2115.1 mg/L to 1992.56 mg/L ($p < 0.05$), compared to T1. This indicated that temperature may play a role in the preservation of phenols, also observed by Zheng et al. (2021).

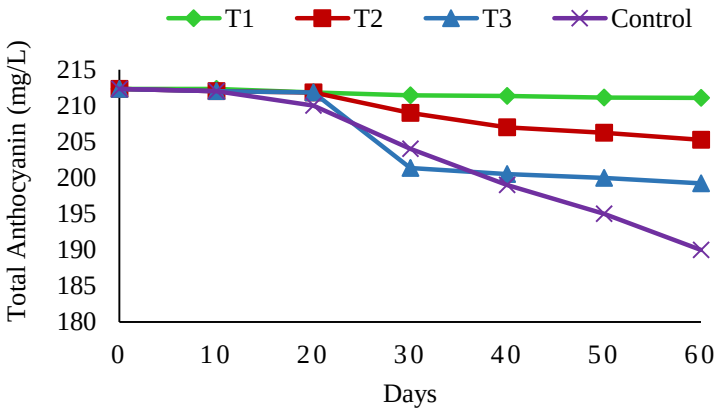

Fig. 6. Change in total anthocyanin in grapes, stored in different conditions.

(T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

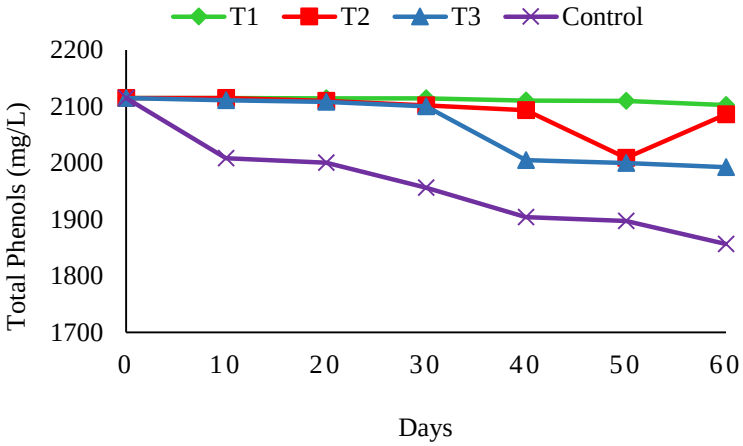

Fig. 7. Change in total phenols in grapes, stored in different conditions.

(T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

Decay % and weight loss % of grapes

Controlled samples were presumably stored without any special conditions or treatments. They experienced the highest decay and weight loss percentages, reaching 78% decay and 33.58% weight loss by the end of the period as shown in Figures 8 and 9. T1 grapes were stored in cold storage with an SO_2 sheet. The decay and weight loss percentages gradually increased over time, reaching 10.78% decay and 15% weight loss by the end of the 60-day period. T2 grapes were stored at room temperature with an SO_2 sheet. The decay and weight loss percentages were slightly higher than the cold storage grapes, reaching 10.9% decay and 15.58% weight loss by the end of the period. T3 grapes were stored in cold storage without an SO_2 sheet. The decay and weight loss percentages were higher than the grapes stored with an SO_2 sheet, reaching 11.15% decay and 16.2% weight loss by the end of the period.

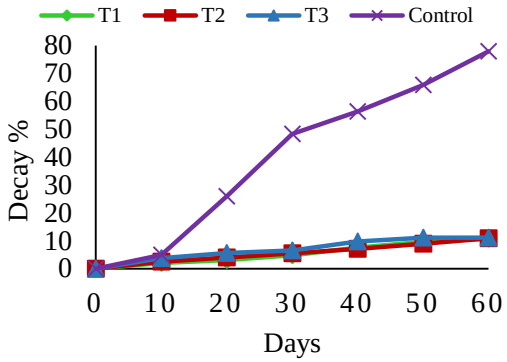

Fig. 8. Decay % of grapes in different conditions. (T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

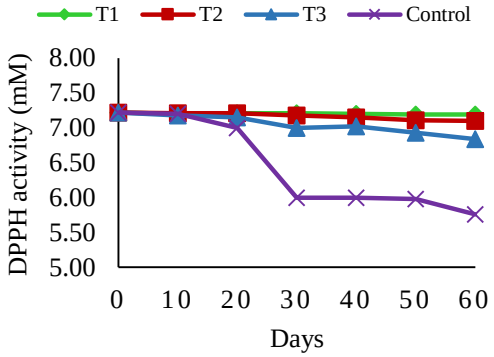

Fig. 10. Antioxidant activity of grapes in different conditions. (T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

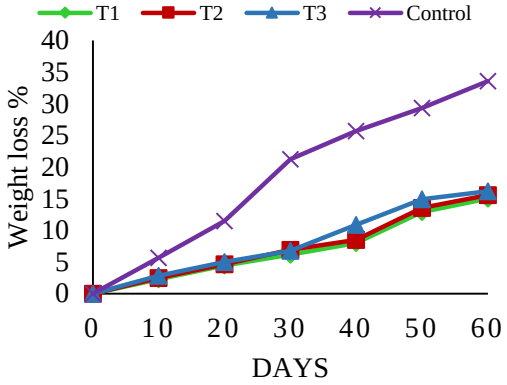

Fig. 9. Weight loss % of grapes in different conditions. (T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

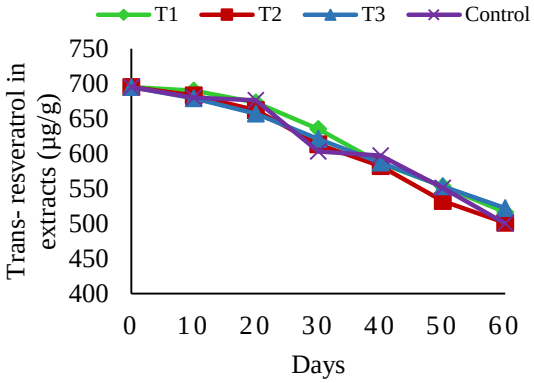

Fig. 11. Trans-resveratrol concentration of grapes in different conditions. (T1 = SO_2 sheet with cold storage grapes, T2 = SO_2 sheet at room temperature grapes, T3 = Without SO_2 sheet at cold storage grapes, Control = Without SO_2 sheet at room temperature grapes).

Antioxidant activity of grapes

The antioxidant activity started at 7.22 mM and decreased to 5.76 mM, stated the highly significant decrease among all conditions as shown in Figure 10. This suggested that without any preservation methods (SO_2 sheet or cold storage), the antioxidant activity of the grapes decreased the most in control sample. The antioxidant activity remained relatively stable in T1 i.e., from 7.19 to 7.22 mM. This suggested that the use of SO_2 sheets in combination with cold storage effectively preserved the antioxidant activity of the grapes. The antioxidant activity started at 7.22 mM and decreased to 7.1 mM in T2. This indicates that while the SO_2 sheet provides some preservation of antioxidant activity, the lack of cold storage led to a slight decreased over time. The antioxidant activity was initially found to be 7.22 mM and decreased more significantly to 6.84 mM ($p < 0.05$) in T3. This suggested that while cold storage alone can preserve some antioxidant activity, the absence of an SO_2 sheet led to a

more noticeable decrease. The antioxidant activity in grapes was primarily due to their phenolic compounds (Nile et al., 2013; Bunea et al., 2012). Higher number of phenolic compounds in T1 contributed to higher antioxidant activity in the storage condition T1 compared to other. The preservation of these compounds can be influenced by storage conditions. SO_2 is known to have preservative qualities and can help maintain the quality of stored grapes. Cold storage can also maintain high antioxidant activity and delay senescence in fruits.

Trans-resveratrol concentration of grapes

The concentration of trans-resveratrol in T1 starts at 695 µg/g and gradually decreased to 516 µg/g over 60 days. This suggested that the synergy of an SO_2 sheet and cold storage reduced the degradation of trans-resveratrol as shown in Figure 11. In T2, the concentration decreased to 501 µg/g over the same period. This indicated that room temperature storage, even with an SO_2 sheet, resulted in a rapid degradation rate of trans-resveratrol compared to cold storage. In T3, the concentration decreased to 522 µg/g over 60 days. This suggested that cold storage without an SO_2 sheet is slightly less effective at preserving trans-resveratrol compared to synergy with an SO_2 sheet. In control samples, the concentration decreased to 500 µg/g over 60 days. This was found to be the highest degradation rate among the four conditions, stated that room temperature without any preservation methods is the least effective at maintaining the concentration of trans-resveratrol.

CONCLUSION

This research investigated the effects of different storage conditions on the quality of grapes. The findings demonstrated that grapes stored with a combination of SO_2 sheets and cold storage (T1) maintained the best overall quality. This treatment resulted in the slowest decline in malic acid content, total soluble solids (TSS), glucose, fructose, trans-resveratrol, decay %, weight loss % and total anthocyanin content, while also exhibited the least significant decrease in total phenols and antioxidant activity. Grapes stored at room temperature without any preservation (control) showed the most significant decline in all measured quality parameters. This highlighted the importance of proper storage techniques to maintain grape quality. The study also found that SO_2 sheets provided some preservative benefits even at room temperature (T2), but these benefits were not as pronounced as when combined with cold storage. Cold storage alone (T3) also offered some preservation compared to room temperature storage, but again, the results were not as effective as the combined SO_2 and cold storage treatment. The research suggested that grape growers and retailers can significantly improve grape quality and shelf life by employing a combination of SO_2 fumigation and cold storage during post-harvest handling.

Conflict of interest

The authors have no conflict of interest to report.

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Influence of an exogenous application of glycine betaine and methionine on biochemical and morphological traits of basils (*Ocimum basilicum* L)

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ABSTRACT

Purpose: This experiment was carried out to examine the impacts of glycine betaine (GB) and methionine (Met) on basil plants' biochemical and morphological traits in two experiments under greenhouse conditions at Guilan University, Iran. **Research method:** Two completely randomized plans were used for the experiment, each involving three replications. The experiment factors during the first experiment were various amounts of GB (0, 50, 100, and 150 mg. L^{-1}), and in the second experiment, we utilized four Met quantities (0, 50, 100, and 150 mg L^{-1}). **Findings:** The results showed that GB utilized at 150 mg L^{-1} led to the maximum leaf fresh and dry weight, stem dry weight, chlorophyll a, chlorophyll b, total antioxidants, and leaf calcium and nitrogen content. The treatments with GB had a 1000 seed weight higher than the control. According to the results, leaf fresh and dry weight, root dry weight, and chlorophyll a and b in control were significantly higher than other Met treatments. Root fresh weight and the florets number per plant in control and 50 mg L^{-1} Met were significantly higher than in other treatments. Besides, the 50 mg L^{-1} Met treatments resulted in higher total phenol, antioxidants, and leaf phosphorus content than the control. **Research limitations:** No limitations were found. **Originality/Value:** The findings of this experiment demonstrate that the use of Met in greenhouse conditions does not have significant effects on basil plants, but GB has significant effects.

INTRODUCTION

Basil (*Ocimum basilicum*) belongs to the botanical family Lamiaceae, as noted by Zangeneh et al. (2019), encompassing a total of 250 genera and an astonishing 7000 species (Pandey et al., 2014). Basil, also known as the genus *Ocimum*, garners significant attention due to its extensive diversity. Approximately 30 species make up its entirety, with a variety that includes herbs and bushes. The distribution of this species spans tropical and sub-tropical regions in Africa, Central Asia, and South America (Singh et al., 2018; Hamoody et al., 2020).

Glycine betaine, otherwise referred to as N, N, N -trimethylglycine, is a preferred compatible solute for many prokaryotes and perhaps the most widely utilized osmolyte in the plant and animal kingdoms (Arafa et al., 2007). As well, GB, as one of the compatible solutes, contributes to salinity by the osmotic adjustment in plants (Ashraf & Harris, 2004), safeguarding the proteins (through sustaining the composition of enzymes such as Rubisco) (Bohnert & Jensen, 1996), protecting the membrane structure (Crowe et al., 1992), Safeguarding cytoplasm and chloroplasts against the detrimental effects of Na^+ (Rahman et al., 2002), Safeguarding the photosynthetic process (Sakamoto & Murata, 2002), and through its role as a sweeper of oxygen radicals (Smirnoff & Cumbes, 1989). The content of GB differs significantly among plant species due to its non-toxic, environmentally safe, and water-soluble nature (Makela et al., 1998). Not all plants can accumulate GB naturally. Although some transgenic plants capable of accumulating GB have been successfully constructed (Sakamoto & Murata, 2002; Sakamoto & Murata, 2000). The association of GB in the regulation of the activities of antioxidant enzymes has been described (Wang et al., 2010). The effectiveness of an exogenous application of GB depends on the type of species, the developmental stage of the plant, the application level, the number of applications, etc. (Ashraf & Foolad, 2007).

Methionine (Met), an essential amino acid, is the primary limiting sulfur amino acid because it can be converted by animals to cysteine, and thus, it meets the requirements for these two amino acids (WHO, 2007). Met is a 4-carbon amino acid synthesized from independently derived components (Kim & Leustek, 2000). Moreover, the sulfur-containing amino acid methionine is a primary metabolite in plant cells. It is a protein constituent and the precursor of S-adenosyl-L-methionine (SAM), the most biological methyl-group donor (Kim & Leustek, 2000). Met serves as a precursor of ethylene in model systems, as well as in fruits and other plant tissues (Lieberman, 1979). In apple tissue, the conversion of methionine to ethylene represents the significant metabolism of the Met (Burg & Clagett, 1967). Met is a precursor of ethylene in several higher, including climacteric tissues of apples, avocados, bananas, and tomatoes (Burg & Clagett, 1967; Lieberman et al., 1966; Mapson et al., 1970). Nitrogen is of vital importance for plant growth due to being a part of amino acid, protein and chlorophyll molecule (Zeraatgar et al., 2019; Roy et al., 2022). In this experiment, we studied the effects of an exogenous GB and Met on the biochemical and morphological traits of basils (*Ocimum basilicum* L.) plants under greenhouse conditions.

MATERIALS AND METHODS

Two completely randomized designs were utilized for conducting the experiments in three replications (with five pots). The experiment factors in the first research were GB (0, 50, 100, and 150 mg L^{-1}), however different amounts of Met (0, 50, 100, and 150 mg L^{-1}) were used in the second one.

Foliar spraying of experimental solutions was done once in the morning every two weeks, starting from the four-leaf stage until the beginning of May. For this purpose, glycine betaine and methionine were thoroughly mixed with water and sprayed entirely on the plant with a

manual sprayer so that the surface of the shoot, stem, and leaf was covered entirely. In each pot, 20-25 mL of the respective solution was employed during every instance of foliar application. On the 6th of January, Isfahan PakanBazr Seed Company cultivated basil seeds that were ready for growth. For the planting process, pots measuring 22 cm in height and 33 cm in diameter were employed.

The seedbed was a combination of cow manure, soil, and sand with a ratio of 2:1:1. Soil organic matter was measured by the hot oxidation method. To determine the number of mineral elements, the soil samples were transported to the laboratory and after drying and grinding the samples, nitrogen was prepared by the more digestible method, and other elements were prepared by the dry digestion method (Hosseini et al., 2021). The amount of absorbed nitrogen was determined by the Kjeldahl method using a micro Kjeldahl device, phosphorus was determined by the Elsen method using a spectrophotometric device at a wavelength of 660 nm, and potassium was determined by the photometric film method (FP7 model). Soil acidity was determined by preparing saturated mud and using a pH meter. The significant properties of the soil utilized within the pot are recorded in Table 1. Fertilizers are not used in the plant growth cycle except for cow manure. Irrigation of the plants was done every three days. After 15 days of greening, the plant thinning process took place to ensure only six plants remained in each pot. The greenhouse temperature during the day and at night was 25°C and 15°C, respectively, while the plant was growing. In addition, the CO_2 concentration measured 350 ppm, the relative humidity stood at 40%, and the photoperiod consisted of 16 hours of light followed by 8 hours of darkness.

On May 4, uprooting four plants from the soil in each pot was conducted to identify several surface and underground vegetative indicators of basil. The estimated indicators included leaf, root, and stem weight (using a scale with an accuracy of one thousandth gram). To determine the dry weight of the root, stem, and leaf the plant samples were placed in an oven at a temperature of 70°C for 48 hours, weighed (Azarmi-Atajan et al., 2023). The height of the plant from the soil surface to the top plant was measured with a ruler (Fani, 2023). Using a ruler with an accuracy of one millimeter, measure the diameter of its main stem. Using the two remaining plants, various reproductive traits were evaluated involving the number of flowers per plant. Pigment content evaluation encompassed chlorophyll a, chlorophyll b, and carotenoid according to the technique detailed in the findings of Minguez-Mosquera and Prez-Galvez (1998). To specify the chlorophyll content, 100 mg of leaf tissue was poured into a microtube, and 1,200 µL of 80% acetone was combined and shaken well. 300 µL of the upper phase of the solution was removed, and 2700 µL of 80% acetone was combined with it, and the absorbance was read with a spectrophotometer at wavelengths of 470, 663.2, and 646.8. The amount of total chlorophyll was calculated using the following equation (1).

$$\text{Chl a} = 12.25\, A_{663.2} - 2.79 A_{646.8} \qquad \text{Chl b} = 21.5 A_{646.6} - 5.1 A_{663.2}$$

$$\text{T Chl} = \text{Chl a} + \text{Chl b} \qquad (1)$$

Table 1. Various soil chemical indicators are utilized for basil cultivation in pots.

Organic carbon (%)	Nitrogen (%)	Calcium (%)	Phosphorous (%)	Potassium (%)	pH	Texture
1.2	1	0.48	0.19	0.55	7.1	Loam Sandy

The method of Jones (2001) was used to calculate the nitrogen, phosphorus, and calcium percentages in leaves. The Bakhshi and Arakava (2006) method was also utilized to define the antioxidant capacity and phenolic content during leaf extract extraction. This way, 2 gr of leaves were chopped separately by a sharp knife. Afterwards, an addition of 4 mL of extraction solvent containing 85% HPLC methanol and 15% acetic acid took place. Following that, the samples were stored at a temperature of 4°C for one whole day and night. In the next step, the samples were poured into a tube and centrifuged at 10,000 rpm for 10 minutes. About 200 µL of the floating phase of each sample was filtered using a 0.45 µm disposable syringe head filter. Phenols were then evaluated using the Folin-Ciocalteau method described by Tavarini et al. (2008). 0.5 mL of plant sample was transferred to the test tube, and after 5 minutes, 0.5 mL of Folin Cicalto was added to it, and then 2 mL of sodium bicarbonate (255 g/L) was added to it and shaken. The solution was kept at room temperature for 10 minutes, and it received an addition of 15 mL of deionized water. Then, it was centrifuged for 40 minutes in a bain-marie at a temperature of 40 degrees Celsius. To achieve this goal, a spectrophotometer with Specifications T80+PG Instrument UV/Vis Spectrometer was used to calculate the absorbance at 760 nm, and the quantity was identified as mg Gallic acid per 100 g of fresh weight (Dorostkar et al., 2022). Sanchez-Moreno et al. (1999) have provided a detailed explanation of the measurement of antioxidant capacity performed using the DPPH free radical scavenging method. For this purpose, 50 µL of the diluted extracts of the samples were poured into tiny test tubes, and 950 µL of standard DPPH 0.1 solution was added to them. The resulting solution was vortexed, and subsequently, it was placed in a dark room at room temperature for half an hour. The control sample included one milliliter of 0.1 standard DPPH solutions. Then, the absorbance of the control and the sample was determined using a spectrophotometer at a wavelength of 517 nm. To end, the use of SAS 9.2 software was employed to perform data analysis, at a 5% probability level, Tukeys HSD test was utilized to determine significant differences among the means.

RESULTS AND DISCUSSION

Vegetative and reproductive growth

Data in Table 2 shows that most morphological and physiological values were significantly increased by an exogenous GB application. Data inTable 3 showed that GB amino acids at 150 mg L^{-1} resulted in an effect with increment in leaf fresh and dry weight, however, the lowest amount was recorded in the control group. Moreover, the maximum and minimum root fresh weights were obtained by 150 and 50 mg L^{-1} GB usage, respectively. Also, the highest stem dry weight was obtained in plants undergoing treatment with 150 mg L^{-1} GB; comparatively, the 0 and 50 mg L^{-1} GB exhibited the lowest levels. In our results, applying external 100 mg L^{-1} of GB produced positive results and enhanced the main stem diameter and the lowest value recorded in the control (Table 3). Data presented in Table 3 shows that GB applied at 50, 100, and 150 mg L^{-1} resulted in the maximum 1000-seed weight, although the minimum amount was reported in the control. In addition, the treatment with 50 mg L^{-1} of GB presented more florets than the control. In general, glycine betaine in plants increases the osmotic potential, and as a result, the cell mass increases with the absorption of water by the plant. The rate at which plants grow and develop is directly influenced by the speed of production and enlargement of new cells and plants can divide cells only in the state of edema, by creating a state of edema by GB cell division increases and plant growth in the state of foliar spraying has caused the matter. The effect of amino acids of GB on the plant height, stem fresh weight, root dry weight, and the number of lateral branches per plant was insignificant (Table 2).

Table 2 demonstrates Met's impact on both vegetative and reproductive growth. The highest values of leaf fresh and dry weight (14.67 and 2.41 g.plant^{-1}) respectively and root dry weight (1.66 g.plant^{-1}) were found in control treatment. Meanwhile, the least values were obtained in 150 mg L^{-1} Met applications. Also, the minimum root fresh weight and the florets number per plant were recorded in 150 mg L^{-1} Met, and the maximum was observed in treatment control and 50 mg L^{-1} Met foliar application (Table 3). Plant height, stem fresh and dry weight, the number of lateral branches per plant, and 1000-seed weight unaffected by Met foliar spray (Table 2). It is likely that the increase in ethylene production is the reason for the decrease in plant growth due to the use of methionine. Morphological characteristics at the reproductive stage, including the number of flowers, significantly increased by an exogenous application of GB on tomatoes (Rezaei et al., 2012). Makela et al. (1998) observed that applying exogenous GB resulted in a substantial enhancement of growth and yield in both greenhouse and field-cultivated tomatoes. Increasing the concentrations of GB by maintaining photosynthetic capacity and the membrane structure improved plant performance (Cha-um et al., 2019). The obtained results were consistent with Seifolahzadeh et al. (2013) findings, who showed that in basil plants, the use of Met resulted in a decrease in plant growth, leading to a reduction in the leaves number, fresh weight, leaf dry weight, and plant height compared to the control. However, our results disagree with Khattab et al. (2016), who compared to the control treatment, the usage of Met in a gladiolus plant showed a significant increment in the number of florets per spike. Correspondingly, Met raised the shoot dry weight in corn (Chen et al., 2005). Likewise, Shekari and Javanmardi (2017) study indicated that Met at 200 mg L^{-1} improved the root dry weight by 177% in Broccoli. Furthermore, the plant growth measurements of squash as expressed by length, fresh and dry weight of the whole plant, and its leaves and shoots are influenced by amino acid treatments (Abd El-Aal et al., 2010). In a recent study, the foliar application of 20 mg/L L-methionine increased the shoot fresh weight, shoot dry weight, shoot length, and root length of Bitter gourd, also known as *Momordica charantia* L, which is a vegetable with a bitter taste (Akram et al., 2020).

Table 2. Mean square on the impact of glycine betaine and methionine on both vegetative and reproductive factors in basil.

Treatment	Variation source	df	Plant height	Main stem diameter	Root fresh weight	Root dry weight	Stem fresh weight	Stem dry weight
Glycine betaine	Treatment	3	40.3^{ns}	1.05*	1.4**	0.12^{ns}	1.19^{ns}	0.31*
	Error	8	30.5	0.23	0.18	0.03	0.98	0.04
	CV (%)	11	10.14	13.21	10.15	9.97	11.17	9.08
Methionine	Treatment	3	88.30^{ns}	0.58**	0.48*	0.09*	1.07^{ns}	0.027^{ns}
	Error	8	30.00	0.05	0.07	0.01	0.70	0.021
	CV (%)	11	11.67	9.67	7.54	8.48	11.44	8.96

Table 2. (*Continued*). Mean square on the impact of glycine betaine and methionine on both vegetative and reproductive factors in basil.

Treatment	Variation source	df	Leaf fresh weight	Leaf dry weight	Number of lateral branches per plant	Florets number	1000- seed weight
Glycine betaine	Treatment	3	4.56**	0.5**	0.11^{ns}	7.22*	0.14*
	Error	8	0.52	0.05	0.41	1.5	0.01
	CV (%)	11	5.17	11.55	20.38	12.04	7.98
Methionine	Treatment	3	8.70*	0.14**	0.08^{ns}	7.63**	0.04^{ns}
	Error	8	1.55	0.01	0.33	0.50	0.01
	CV (%)	11	9.58	6.64	23.89	10.74	6.42

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

Table 3. Means comparison of the effect of glycine betaine on several factors for vegetative and reproductive growth of basil.

Treatment	Concentration (mg L^{-1})	Plant height (cm)	Main stem diameter (mm)	Root fresh weight (g.plant^{-1})	Root dry weight (g.plant^{-1})	Stem fresh weight (g.plant^{-1})	Stem dry weight (g.plant^{-1})
Glycine betaine	0	50^{a}	3.02^{b}	3.9^{ab}	1.66^{a}	8.63^{a}	2.02^{b}
	50	53.3^{a}	3.57^{ab}	3.53^{b}	1.87^{a}	8.13^{a}	2.04^{b}
	100	55.6^{a}	4.46^{a}	4.28^{ab}	2.08^{a}	9.36^{a}	2.57^{ab}
	150	58.6^{a}	3.61^{ab}	5.12^{a}	2.08^{a}	9.46^{a}	2.61^{a}
Methionine	0	53.66^{a}	2.96^{a}	4.03^{a}	1.66^{a}	7.96^{a}	1.65^{a}
	50	48^{a}	2.86^{a}	3.95^{a}	1.56^{ab}	7.67^{a}	1.69^{a}
	100	45.33^{a}	2.19^{a}	3.73^{ab}	1.38^{ab}	7.12^{a}	1.72^{a}
	150	40.66^{a}	2.11^{a}	3.14 b	1.26^{b}	6.61^{a}	1.5^{a}

Table 3. (*Continued*). Means comparison of the effect of glycine betaine on several factors for vegetative and reproductive growth of basil.

Treatment	Concentration (mg L^{-1})	Leaf fresh weight (g.plant^{-1})	Leaf dry weight (g.plant^{-1})	Number of lateral branches per plant	Florets number	1000- seed weight (g)
Glycine betaine	0	13^{b}	1.5^{b}	3^{a}	8.33^{b}	1.42^{b}
	50	13.5^{ab}	1.87^{ab}	3^{a}	12^{a}	1.8^{a}
	100	13.84^{ab}	2.18^{ab}	3.33^{a}	9.66^{ab}	1.91^{a}
	150	15.82^{a}	2.46^{a}	3.33^{a}	10.66^{ab}	1.83^{a}
Methionine	0	14.67^{a}	2.41^{a}	2.33^{a}	7.33^{a}	1.67^{a}
	50	13.79^{ab}	2.06^{ab}	2.66^{a}	8.33^{a}	1.67^{a}
	100	12.92^{ab}	2.02^{ab}	2.33^{a}	6^{ab}	1.53^{a}
	150	10.7^{b}	1.88^{b}	2.33^{a}	4.66^{b}	1.43^{a}

According to Tukey's test, there isn't a significant difference ($P \leq 0.05$) between means that have the same letter (s) within a column.

Pigments content

As shown in Table 4, foliar spray application of GB treatments led to a significant increase in chlorophyll a and b content. Data in Figure 1 show that GB foliar application increased chlorophyll by 25.96% compared to control plants. Also, chlorophyll b at 150 mg l^{-1} concentration was 32.83% more than the control, but there was no significant carotenoid content. GB can enhance the biosynthesis of chlorophyll or prevent it from breaking down or slowing down its decomposition. It seems that increasing the concentration of glycine betaine has caused an internal increase of the choline precursor in the leaf and has prevented the destruction of chlorophyll and the activity of the chlorophyllase enzyme; therefore, it has increased the amount of chlorophyll a, and b is added.

Data presented in Table 4 and Figure 2 show that chlorophyll a and b in control was significantly higher, while the lowest was related to the 150 mg L^{-1} Met treatment. Moreover, the foliar Met application did not change carotenoid content significantly.

The application of GB externally increased chlorophyll contents and subsequently improved the drought tolerance in wheat plants (Raza et al., 2015). Chlorophyll a and b concentrations in the leaf tissues treated with 200 mM of GB significantly degraded by 43.67% and 45.19%, respectively, compared to those without foliar spray treatment (Cha-um & Kirdmanee, 2010). An exogenous application of GB concentrations appears to increase internal precursor choline in leaves prevent chlorophyll degradation and inhibit the activity of the chlorophyllase enzyme. Therefore, chlorophyll concentration increased in the leaf (Miri & Mohammad, 2013). Hence, applying GB in different concentrations resulted in the accumulation of internal GB, thus improving the chlorophyll b content. The role of GB is preserving and regulating osmotic, maintaining the integrity of the plasma membrane, and maintaining the fourth building of protein by increasing the accumulation of chlorophylls and

the absorption of carbon dioxide, facilitating the electron transfer and protecting the activity of proteins and fatty mucous membrane of thylakoid in the photosystem II can be as one from physiological factors to stresses (Murata et al., 1992).

By the findings of Seifolahzadeh et al. (2013), it has been proven that the chlorophyll and carotenoid content of the basil plant decreased as Met was applied. Also, data showed that the highest chlorophyll values in snap beans were found with Met (200 mg L^{-1}) in the initial season and by Met (100 mg L^{-1}) in the second season. The highest values of chlorophyll b were found in the control treatment in the first season and by Met at a low concentration (100 mg L^{-1}) in the second one (El-Awadi et al., 2011). Moreover, Met at the concentration of 200 mg L^{-1} showed the highest chlorophyll content in Broccoli seedlings (Shekari & Javanmardi, 2017). Also, the amino acid Met increased the efficiency of the photosynthesis process by increasing the leaf pigments (chlorophyll a, b, and carotenoids) in the Gladiolus plant (Khattab et al., 2016).

Table 4. Mean squares for the influence of glycine betaine and methionine on pigment content factors in basil.

Treatment	Variation source	df	Chlorophyll a	Chlorophyll b	Carotenoid content
Glycine betaine	Treatment	3	0.03**	0.02**	0.001^{ns}
	Error	8	0.003	0.003	0.001
	CV (%)	11	5.23	7.41	6.35
Methionine	Treatment	3	0.038**	0.01*	0.0006^{ns}
	Error	8	0.004	0.002	0.001
	CV (%)	11	7.14	8.05	7.69

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

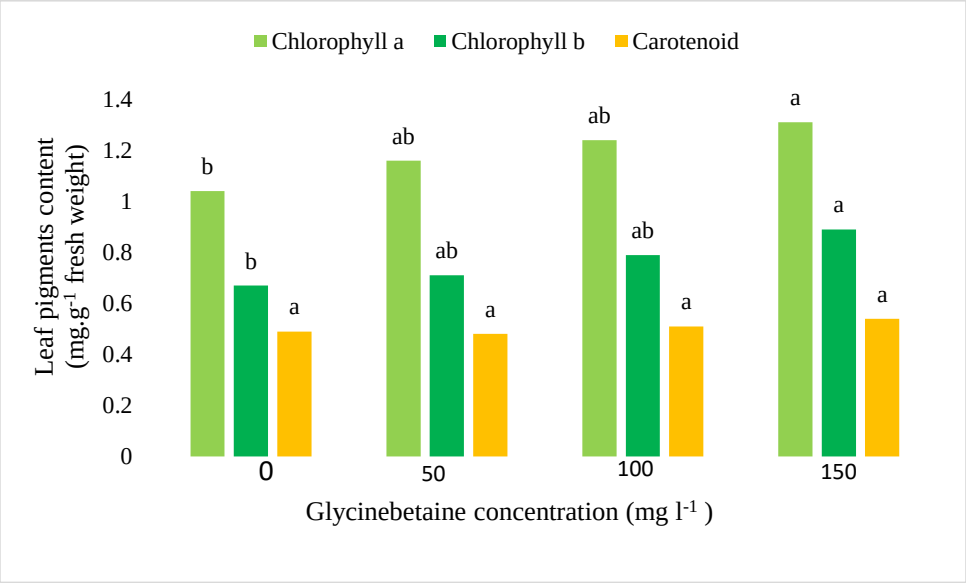

Fig. 1. Evaluating the impact of multiple levels of glycine betaine on the content of leaf pigments in basil.

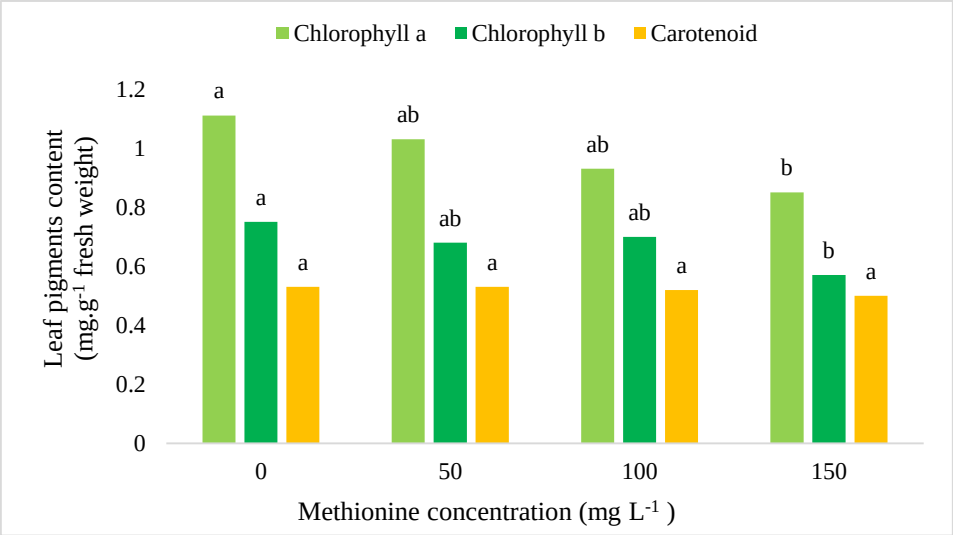

Fig. 2. The impact of varying methionine levels on basil leaf pigment content.

Nutrients content

Based on the results of Table 5, GB levels significantly affected the N and Ca content of the leaf. Thus, the highest N and Ca content in the leaf occurred at the 150 mg L^{-1} GB application level. Also, GB applications significantly affected the content of P in the leaf. Therefore, plants treated with a 50 mg L^{-1} GB usage level demonstrated the most significant P content in the leaf, while the minimum related to the 150 mg L^{-1} GB was 0.31% (Fig. 3).

The effect of Met foliar spray on nutrient content is presented in Table 5. The amino acid of methionine (100 mg L^{-1} Met) also significantly increased the leaf P content of the basil, which, averaged across rates, was 25.80% surpassing the control value. However, the result showed no significant effects of the Met application on leaf N and Ca content (Fig. 4).

The use of exogenous GB significantly improved the uptake of N, P, and Ca in plant wheat (Raza et al., 2015). This result agrees with those observed by Abd El-Aal et al. (2010), who showed that the chemical properties (P) of squash fruits were influenced by the foliar spraying of amino acid compounds during the seasons of 2006 and 2007. As well as, treatment with Met raises the absorption of nitrogen, phosphorus, and potassium in corn (Chen et al., 2005). Because Met is involved in auxin synthesis and because auxin promotes root initiation, it can help the plant absorb more nutrients (Shekari & Javanmardi, 2017).

Table 5. Mean squares for the influence of glycine betaine and methionine on leaf nutrient factors in basil.

Treatment	Source of variation	df	Leaf nitrogen content	Leaf phosphorous content	Leaf calcium content
Glycine betaine	Treatment	3	0.36^{**}	0.003^{**}	0.03^{*}
	Error	8	0.04	0.0004	0.008
	CV (%)	11	6.14	5.83	8.88
Methionine	Treatment	3	0.04^{ns}	0.003^{**}	0.004^{ns}
	Error	8	0.02	0.0003	0.008
	CV (%)	11	5.15	5.48	8.68

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

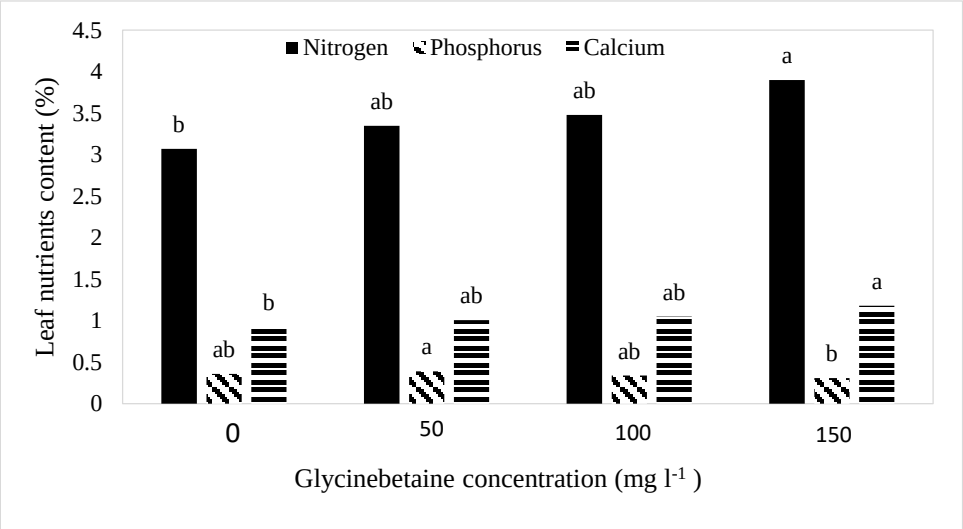

Fig. 3. The impact of various levels of glycine betaine on the nutrient content of basil leaves.

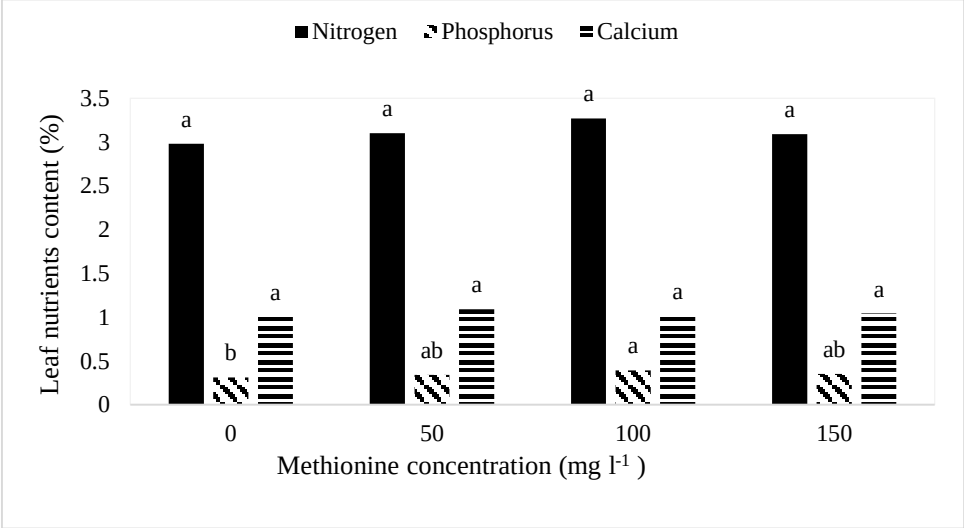

Fig. 4. Investigating the correlation between different methionine concentrations and basil leaf nutrient levels.

Total phenol and antioxidants

Data in Table 6 shows that total phenol and antioxidants are significantly affected by GB and Met applications. GB applications beyond the 50 mg L^{-1} GB treatment increased the total phenol compared to the control (Fig. 5). Also, treatments with 50 mg L^{-1} GB resulted in higher antioxidants than the control (Fig. 6). As shown in Figures 7 and 8, the highest total phenol and antioxidants were obtained when Met applied at 100 mg L^{-1} to the plants.

Under the condition of high salinity stress, it has been reported that GB has shown an increase in antioxidant enzyme activities in rice, maize, and mung beans (Hasanuzzaman et al., 2014; Hossain & Fujita, 2010; Nawaz & Ashraf, 2010). Shams et al. (2016) reported that the total phenolic content in the lettuce plants treated with 25 mM GB was significantly increased.

A similar result was also described by Seifolahzadeh et al. (2013), who showed that using Met has an inhibitory effect on the antioxidant enzyme activity, including polyphenol oxidase and peroxidase. Also, Met leaf treatment increased the phenolic compounds in the basil plant.

Table 6. Mean squares for the impact of glycine betaine and methionine on various biochemical parameters in basil.

Treatment	Variation source	df	Total antioxidants	Total phenol
Glycine betaine	Treatment	3	18.96**	28.38*
	Error	8	1.33	4.67
	CV (%)	11	3.25	4.86
Methionine	Treatment	3	8.26**	16.61*
	Error	8	0.38	3.59
	CV (%)	11	1.75	4.23

ns = non-significant, * and ** represent significance at the 5% and 1% probability levels, respectively.

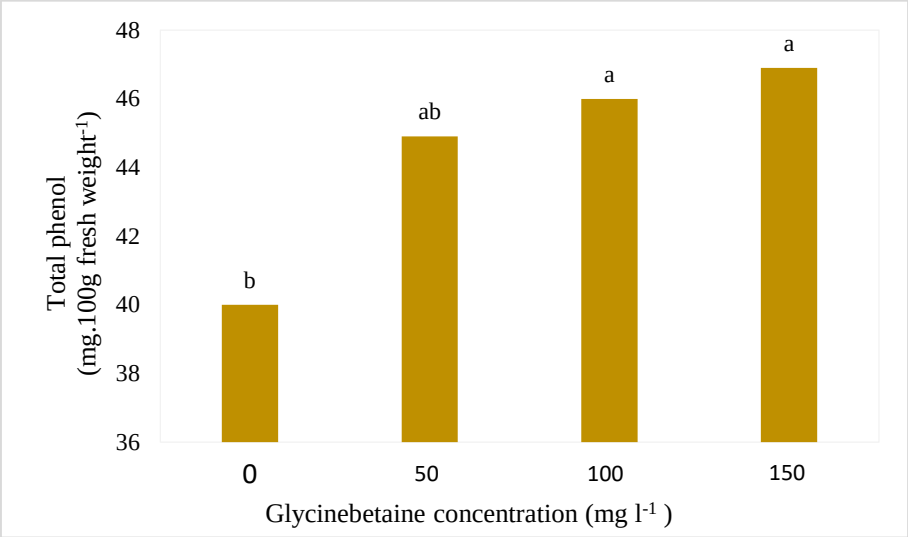

Fig. 5. Impact of various glycine betaine levels on the total phenolic compounds found in basil.

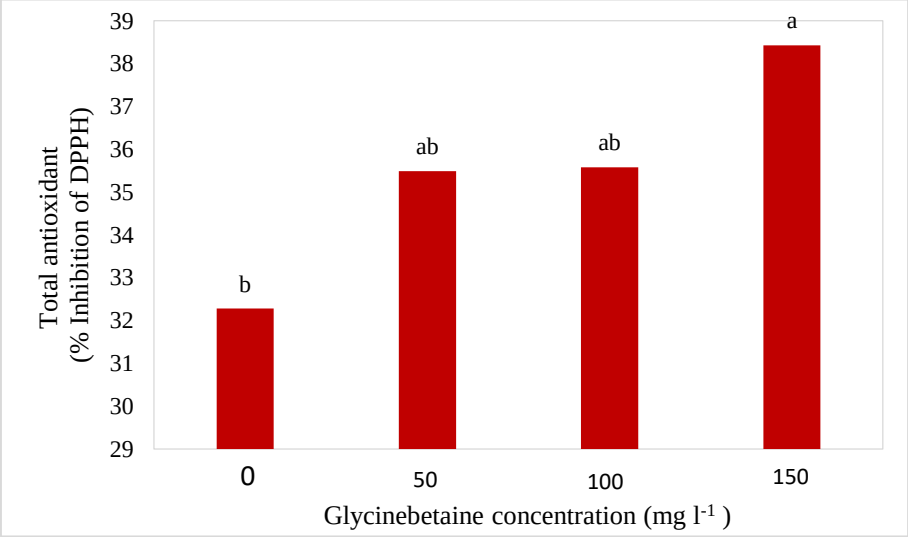

Fig. 6. The influence of various concentrations of glycine betaine on the total antioxidants found in basil.

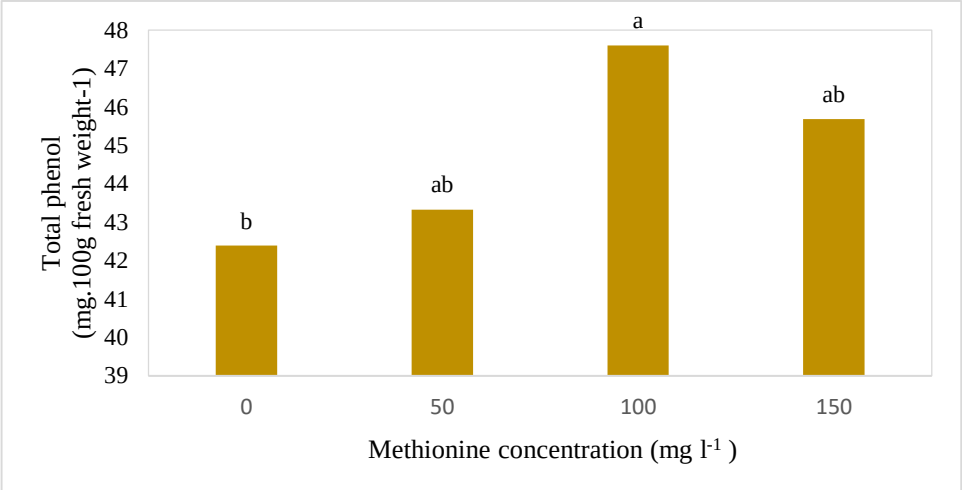

Fig. 7. The impact of different methionine levels on basil total phenolic compounds.

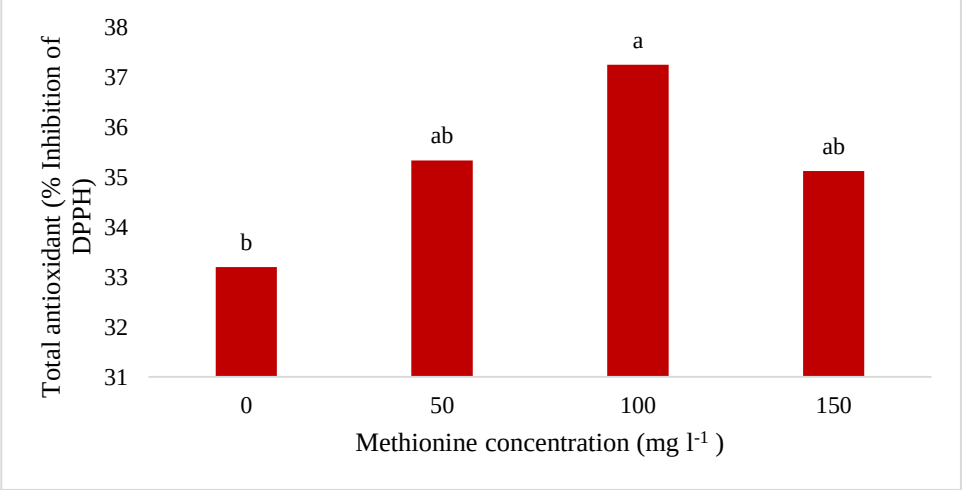

Fig. 8. The impact of different methionine levels on basil's total antioxidant content.

CONCLUSION

GB at a concentration of 150 mg.L^{-1} significantly affected the biochemical and morphological traits (stem's dry weight, root's fresh weight, leaf's fresh and dry weight, content of nitrogen and calcium in leaves, pigments chlorophyll a and b, and total antioxidants). The positive influence of amino acids can be attributed to several reasons. Initially, amino acids maintain the structure of proteins required for cell division. Then, amino acids help the division and growth of plant cells by entering the hormonal structures and, finally, the ability of amino acids to convert into polyamines that function in cell division, differentiation, and growth. GB is a quaternary amine, and it is made as a compatible substance and osmolyte in some plants and has a unique protective effect on the organs, biological membranes, proteins, and cellular enzymes. However, some plants are not able to synthesize this material under stress and non-stress conditions, so researchers are trying, or using genetic manipulations, to enable these plants to synthesize GB or exogenously use this material on plants, particularly on plants that cannot synthesize GB, it improves growth. Previous studies have proven that amino acids can directly or indirectly affect plant physiological activities.

Conflict of interest

The authors declare that they have no conflict of interest.

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Recycling of sawdust waste as biodegradable active gelatin films against *Aspergillus flavus,* a field-borne pathogen in garlics (*Allium sativum* Linn.)

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ABSTRACT

Purpose: Sawdust, a by-product of wood workplaces, poses environmental contamination and reduces workspace efficiency. This research aimed at recycling sawdust from rain tree by incorporating its extracts into gelatin films to create active films with antifungal properties against *Aspergillus flavus*. **Research method:** Sawdust was extracted by microwave with various solvents and electrical powers. The extract (0, 0.25, 0.5, 1, and 2%) were then tested for *A. flavus* inhibition. The extract was also incorporated with gelatin for making wrapped films and tested for inhibition potential on garlic inoculated with *A. flavus*. **Findings:** The optimal microwave extraction condition utilized a solvent mixture comprising distilled water and 95% ethanol in a 1:1 v/v ratio, applying 100 watts of electrical power for 30 seconds, repeated 5 times. This method yielded 23.26 mg/g of tannin. Furthermore, the 2% concentration of the extract significantly inhibited both mycelium growth and spore germination of *A. flavus* ($P \leq 0.05$) when tested on a petri dish. Additionally, incorporating 2% of the crude extract into gelatin film resulted in the most favorable outcome. This treatment demonstrated the capability to prolong the shelf life of wounded-inoculated garlic for more than 12 days. **Research limitations:** No limitations were found. **Originality/Value:** Sawdust originating from a rain tree can be recycled biodegrable actine gelatin films against *A. flavus*, a field-bomepathogen in garlic.

INTRODUCTION

The volume of sawdust from wood carving areas continues to increase due to a rise in demand for wood products. Ban Luk Tai Village is a wood handicraft village in Lampang province, the northern part of Thailand. Most of the villagers have a career in wood carving. Many items, like mortars and pestles, tables, home decoration accessories and wood games, are made from rain tree wood. The villager has not fully exploited the potential for recycling these waste materials, and therefore they are accumulating, causing less work space and starting to rot. Sustainable and applicable methods for enhancing sawdust usage must be looked for.

Several studies have shown that various parts of the rain tree have phytochemical substances that show antioxidant, antibacterial, insecticidal, antifungal and cytotoxic effects (Prasad et al. 2008; Ukoha et al., 2011; Vinodhini & Rajeswari, 2018; Boonkorn et al., 2020). Ukoha et al. (2011) suggest that ground pods of *S. saman* could be a significant source of natural antimicrobials and antifungals, such as tannins. The incorporation of extracted from rain tree sawdust with wrapping film is interesting because plastic films are highly concerned as they are harmful to the environment. Gelatin, a product made from animal protein, is a kind of edible-biodegradable polymer that has potential for the manufacturing of food packaging applications (Chen et al., 2019). Wang et al. (2017) added different concentrations of tannins to the gelatin solution and found that the tannin-gelatin film showed potential development value in the field of food packaging.

Garlic (*Allium sativum* L.) is classified within the Alliaceae botanical family and ranks as the second most commonly utilized *Allium* variety after onion (Amerian et al., 2024). Garlic serves as both a culinary ingredient and a medicinal herb (Ayed et al., 2019), commonly used in various Thai dishes. Presently, garlic sold in stores is often prepared by cutting into bulbs and packaging in mesh bags, which typically have a short shelf life due to being susceptible to destruction by the *A. flavus* fungus present on the garlic skin since cultivation. Therefore, peeling the garlic and packaging it in trays covered with easily biodegradable plastic, containing natural extract compounds capable of inhibiting the growth and germination of fungal spores, can be a method that helps extend shelf life for storage and distribution without the use of chemical substances.

For all the reasons mentioned above, the developed natural biodegradable films with rain tree extracts can release phytochemicals into the agricultural products or food surfaces. This can increase the stability of the products or prolonging the storage life of crops. It is also friendly to the environment. During this study, the microwave-assist extraction of sawdust waste was optimized, and the inhibition efficiency of field-borne pathogen in garlic, *A. flavus*, was investigated *in vitro*. The crude extract was also incorporated into the gelatin film to form an active gelatin film, and *in vivo* testing with garlic was performed.

MATERIALS AND METHODS

Microwave-assist extraction of the plant materials

Sawdust waste from rain tree (*Samanea saman* (Jacq.) Merr.) was obtained from Ban Luk Tai Wood Handicraft Village in Lampang province, Thailand. Dried, finely ground sawdust (10 g) was taken in a glass flask of approximately 250 ml capacity. A hundred ml of three extraction solvents, including distillate water, distillate water: 95% ethanol (1:1), or 95% ethanol was added and compared. The flasks were then subjected to microwave (Model MS20A3010A, Sumsung, Korea) and each of the three electrical power treatments was applied, including 100, 300, or 450 watts. Plant materials were irradiated in a microwave for

30 s through five consecutive extraction cycles. For each cycle, the extract was allowed to cool to room temperature for 2 min before the next cycle begin. The temperature of the finishing cycle was recorded and then the content of the glass flask was subjected to centrifugation for 10 min at 3000g at 4°C. The supernatant was dried at 50°C until weight stability, and then the crude extract was collected and weighted.

The basic chemical characteristics of tannin in the crude extracts were determined by the interaction with 1% Iron chloride ($FeCl_3$), 1%bovine serum albumin (BSA), and 1% lead acetate ($Pb(C_2H_3O_2)_2$) following the methods of Moosophin et al. (2010) and Elgailani and Ishak (2016). The presence of tannin in the extract made white precipitation with BSA, dark red precipitation with $Pb(C_2H_3O_2)_2$ and black precipitation with $FeCl_3$ which considered positive. Quantifying tannin in the crude extracts was performed using the Folin-Ciocalteu method, followed by the methods of Ukoha et al. (2011). Absorbance was measured with a UV/Visible spectrophotometer (Model Velocity 18R, Dynamica Scientific Ltd., UK) at 700 nm. The tannin content was expressed in terms of mg/g of tannic acid. All the determinations of tannin in the solutions were carried out in triplicate. The best extraction condition will be chosen for the inhibition efficiency test on the food-borne pathogen in garlic, *A. flavus*, both *in vitro* and *in vivo*

Inhibition efficiency test of crude extracts on *A. flavus* (*in vitro*)

A. flavus strain was provided by the microbial laboratory of the Science Faculty, Lampang Rajabhat University. The microbial were inoculated on potato dextrose agar (PDA) solid medium for 7 days at 25°C, and then their mycelium was cut and cultured on a newly prepared PDA petri-dish every 3 days for 21 days. To conduct the *in vitro* inhibitory test on mycelium growth, we followed the protocol of Masiello et al. (2019) with slight modifications. The crude extract was incorporated into warm liquid PDA medium to achieve concentrations of 0, 0.25, 0.5, 1, and 2% (w/v). As a positive control, we employed 1% benomyl fungicide in PDA. After solidification of the tested medium, the same age mycelium discs along the edge of *A. flavus* colony were cut with a sterile cork borer (0.4 cm diameter), and each one was placed on the surface of a PDA plate, 5 plates per treatment. The mycelium was incubated in dark at 25 °C for 7 days. Mycelium diameter was determined every day for 7 days. For the *in vitro* inhibitory test on spore germination, we followed the protocol of Hu et al. (2013) with slight modifications. Spores of *A. flavus* were washed from a 7-day-old *A. flavus* growth on a PDA plate and suspended in sterile distilled water to produce a final concentration of 1×10^6 spore's ml^{-1}. Twenty microliters of the spore suspension was poured and spread on the surface of the PDA plate containing crude extract of each treatment as described above, 5 plates per treatment. The number of colony-forming units (cfu) ml^{-1} was determined at 0, 24, 48, and 72 h after the incubation in dark at 25°C.

The incorporation of crude extracts into gelatin films

The active gelatin films were prepared by casting process as described by Etxabide et al. (2022) with slight modifications. A hundred ml of distilled water was mixed with 3.5 g of beef skin gelatin (Gelatin 160 Bloom, Australia) under continuous stirring at 2000 rpm, 50°C until completely dissolved. Then, 4 ml of glycerol was added and stirred for 5 min. Finally, the crude extract was added to the film solution to obtain the concentration of 0, 0.25, 0.5, 1, and 2%. From the preliminary study, adding crude extract of more than 2% caused obvious uneven porous on the film surface. For these reasons, 0-2% of the crude extract was chosen for this study. The positive control was 1% benomyl fungicide. The mixtures were stirred for 15 min to obtain a homogeneous film solution. The 10 mL of each film solution treatment

was then applied on a 170×120×5 mm glass plate and kept in a desiccator until dried. Film thickness was measured by a hand-held micrometer (RS PRO, Thailand).

Film color was measured by colorimeter (Model F50, FLUXANAR, Germany) and expressed as L* a* and b* values. The morphology of the film was examined by light microscope (Model CX22, OLYMPUS, Japan). For all determinations, five measurements were taken at random positions on each film sheet, 5 sheets per treatment. For testing the mechanical properties of the tested films, the tensile strength and elongation at break for each film were measured by using a digital push-pull gauge (HF-2 Model, ABALLTECHNO Co., Ltd., China). Ten specimen samples for each tested film were subjected to the analysis. The time to the decomposition of the film was also determining, all treated films were placed on dry soil in a ventilated room at room temperature (35±2°C).

Inhibition efficiency test of the gelatin films on *A. flavus* in garlics

Garlic bulbs (*Allium sativum*) grown under standard cultural practices were harvested at the commercial maturity stage from an orchard in Mae Hong Son, Thailand in March 2021. The uniform and non-damage bulbs were selected, and their peel was gently unwrapped, surface sterile with 75% ethanol, and dried for 30 min at room temperature. They were separated into 2 groups: wounded inoculated and non-wounded inoculated with *A. flavus* spores. For the wounded-inoculated group, the bulbs were artificially wounded with a sterile needle (1 mm depth), and then a droplet (5 µl) of the spore suspension at a concentration of 2.2×10^6 spores ml^{-1} was placed on that needle-made wounds and then dried for 30 min at room temperature.

The non-wounded inoculated group, a droplet of the spore suspensions was placed directly on the bulb surface. Five pieces of garlic bulb were placed in each paper tray, 3 trays per treatment, and then tightly covered with gelatin films with varying crude extract concentrations. All samples were stored at room temperature (35±2°C). The disease incidence was inspected every two days. Bulbs showing brownish spots on the surface with white mycelium and greenish spores were considered infected pieces.

Statistical analysis

The experimental setup was arranged in a completely randomized design (CRD). Data were subjected to two-way analysis of variance (ANOVA) using SPSS software (Trial version). Duncan's multiple range tests were used to determine significant differences between treatments at a 95% confidence interval.

RESULTS AND DISCUSSION

The microwave-assist extraction method was used in this study. This novel green technology is non-thermal, quick, give high extraction yield, low solvent and time consuming, do not use any hazardous chemicals, and ensures the stability of thermolabile components in contrast to other extraction technique (Bagade & Patil, 2021; Kayahan & Saloglu, 2021; Antony & Farid, 2022). Das et al. (2020) stated that tannin in plant materials can be extracted with water alone or water with other solvents, and several advanced technologies such as microwave or ultrasonication had shown to extract tannins efficiently.

From the study, the appropriate solvent for extracting sawdust was the mixture of distillate water: 95% ethanol (1:1) at electrical power of 100, 300, and 450 watts (Table 1). The condition gave statistically ($P\leq0.05$) higher extraction yield and tannin concentration than other conditions. No difference was found between the three electrical powers of the same solvent. All tested extraction conditions gave a final temperature of the extracts not more than 60 °C which is all in good ranges because at high temperatures, thermal degradation of

important substances in the plant materials may occur. From basic chemical tests of the crude extract, the interaction with metal ions such as $FeCl_3$ and $Pb(C_2H_3O_2)_2$ and the ability to precipitate BSA indicated the presence of tannin (Moosophin et al., 2010; Elgailani & Ishak, 2016).

The finding was in accordance with Moosophin et al. (2010) who found that a solvent mixture of water and 95% ethanol (1:1 v/v) gave the highest yield of tannin from mangosteen peel extract. Meanwhile, the yield of tannin reported in this study was about two times higher than reported by Boonkorn et al. (2020) who extracted tannin from rain tree sawdust by using 95% ethanol at 80°C. Das et al. (2020) report the success of various kinds of solvent used in tannin extraction from plants such as water, acetone, methanol, ethanol, sodium sulfite, and NaOH. The different results of extraction yield might be related to the processing parameters such as plant species, raw materials, particle size, temperature, and time.

For the next antifungal test of crude extracts against *A. flavus*, the appropriate condition to extract sawdust was used. As a result, solvents of distillate water: 95% ethanol (1:1) and electrical power at 100 watts were selected. Crude extract from rain tree resulted in significantly reduced mycelium disc diameter of *A. flavus* (Table 2). Increasing the concentration of the crude extracts in the PDA plate resulted in a significant reduction in the mycelium disc diameter of *A. flavus.* The concentration of 1 and 2% could obviously suppress, although could not totally inhibit, the mycelium growth when compared to the control, 0.25 and 0.5%. While 1% benomyl can completely inhibit the growth of mycelium as it is a highly effective fungicide.

Incorporated crude extract in the PDA plate also significantly inhibited the spore germination of *A. flavus* compared to the control. The number of colony-forming units of the fungi on the PDA plate was considerably reduced after exposures from 0.25 up to 2% of the crude extract. At 72 h after incubation, germinated spores were visually noticeable in the control, 0.25%, and 0.5% treatment with the recorded fungal concentrations of 8.82×10^5, 6.31×10^5, and 7.11×10^5 cfu ml^{-1}, respectively. Meanwhile, a significantly reduced of spore germination was found in the 1 and 2% treatments with the average value of 4.68×10^5 and 1.32×10^5 cfu ml^{-1}, respectively. The results were in accordance with various works that found highly significant antifungal activity from the rain tree extract (Prasad et al., 2008; Vinodhini & Rajeswari, 2018; Boonkorn et al., 2020).

Table 1. Percent yield, tannin content, and basic chemical tests of the extract from sawdust those extracted with various conditions.

Solvents	Electrical power (watts)	Final temperature (°C)	Percent yield (%)	Chemical tests			Tannin (mg g^{-1})
				1% $FeCl_3$	1% BSA	1% $Pb(C_2H_3O_2)_2$	
distillate water	100	38^{c}	1.12^{b}	+	+	+	10.78^{c}
	300	47^{bc}	1.40^{b}	+	+	+	19.29^{ab}
	450	51^{b}	1.27^{b}	+	+	+	20.19^{ab}
distillate water: 95% ethanol (1:1)	100	39^{c}	2.83^{a}	+	+	+	23.26^{a}
	300	49^{bc}	3.11^{a}	+	+	+	22.96^{a}
	450	58^{a}	3.17^{a}	+	+	+	23.50^{a}
95% ethanol	100	39^{c}	1.48^{b}	+	+	+	16.57^{b}
	300	54^{ab}	1.78^{b}	+	+	+	21.93^{ab}
	450	58^{a}	1.72^{b}	+	+	+	21.49^{ab}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$), and the symbol "+" means "positive" in chemical tests (i.e. presence of tannin in the extract).

Germination of fungal spores is a vital step in the pathogenesis of *A. flavus* and any effect on their germination may have a corresponding effect on the disease severity. It appeared that direct contact of fungal spores to the PDA plate with crude extract of rain tree at high concentration (1-2%) could partly inhibit the fungal spore germination, possibly due to lethal action on the spores. The ability of tannins to disrupting the fungal cell membrane structures is well known. Two mechanisms of fungicidal effects by tannin were described by Carvalho *et al.* (2018) including (i) tannin binds to ergosterol in fungal membrane and then make pores in the structure or (ii) tannin inhibits enzymes involved in the ergosterol synthesis. Moreover, the tannin in the rain tree extracts can precipitate protein in the microbial cell, according to the ability to precipitate BSA protein as was shown in this experiment. Denature of the cell membranes and protein precipitating might be the reasons for the inhibition of mycelium growth and spore germination in *A. flavus* in this study.

Active gelatin films incorporated with various concentrations of sawdust crude extract were made by casting technique. The color measurement (L*, a* and b*) of the treated films was shown in Table 3. Higher concentrations of the crude extract tended to decrease L* and increased a* and b* values than lower concentrations when compared to control or benomyl treatments. This indicated that when the extract concentration ranging from 0.25 to 2% was used, the film was darker and more reddish and yellowish. These were most probably due to the brownish color of the sawdust extracts. Accordance to Peña et al. (2010) who found that gelatin films incorporated with tannin turned from light yellow to brownish color as tannin content increased.

Film thickness tended to increase with increasing the extracts or benomyl, but no statistical difference. The increasing solids content in the polymer matrix of the films enhances the film thickness layer (Said & Sarbon, 2022). Tensile strength values of the treated gelatin films were also examined, it was 19.36 MPa in non-treated gelatin films (0% crude extract) and was gradually decreased to 13.11 MPa by incorporating sawdust extracts up to 2%. The free volume of the film matrix weakens its structural stability, thus may be the reason for the lowering of the film's tensile strength. The elongation at break values of the treated films also decreased with an increase in the sawdust extract concentration, reflecting the decreased flexibility and elongation potential of the films. The gelatin films in this study showed higher values of tensile strength and elongation at break than those reported by Jirukkakul (2022). The difference in gelatin source and other ingredients that corporations use in the films may be the reasons.

Table 2. Inhibition efficiency on *A. flavus* of the crude extracts from rain tree sawdust at the various concentrations compared to benomyl.

Treatment	Mycelium diameter (cm)	Spore germination ($\times 10^5$ cfu ml^{-1})
0%	7.60^{c}	8.82^{a}
benomyl	0.00^{d}	0.00^{e}
0.25%	5.80^{a}	6.31^{b}
0.50%	5.58^{a}	7.11^{b}
1%	3.60^{ab}	4.68^{c}
2%	3.30^{b}	1.32^{d}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

Table 3. Property determinations of gelatin films incorporated with various concentrations of the crude extracts or benomyl.

Treatment	Color measurements			Thickness (mm)	Mechanical properties	
	L*	a*	b*		Tensile strength (Mpa)	Elongation at break (%)
0%	91.85^{a}	-0.83^{d}	5.11^{d}	0.04^{a}	19.36^{a}	105.20^{a}
Benomyl	85.03^{c}	-0.36^{c}	7.10^{b}	0.08^{a}	10.15^{c}	58.77^{b}
0.25%	90.51^{a}	0.04^{b}	5.50^{cd}	0.04^{a}	15.23^{b}	82.80^{ab}
0.50%	90.18^{ab}	0.05^{b}	5.88^{bcd}	0.04^{a}	15.88^{b}	83.13^{ab}
1%	89.87^{ab}	0.31^{b}	6.81^{bc}	0.06^{a}	14.32^{b}	83.02^{ab}
2%	88.03^{b}	1.08^{a}	10.34^{a}	0.07^{a}	13.11^{b}	61.57^{b}

Note: Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

The surface of the active gelatin films incorporated with varying concentrations of the extract was evaluated using a transmitted light microscope, which demonstrated a uniformity matrix only in the control. Increasing the extracts or benomyl showed partial granules in the film matrix with a particle size of approximately 10–50 μm. The small granules in the film matrix lead to a decrease in tensile strength and elongation at break values, as mentioned above. The incomplete dissolution of the materials in the gelatin film matrix was found, in accordance with the finding of Hanani et al. (2019) who found the same characteristics of the films when pomegranate peel was incorporated into gelatin films.

After wounded inoculating garlic with *A. flavus* and then wrapped with the tested films, disease incidence on garlic first appeared on the fifth day of storage at 35±2°C. Symptom of the fungal attack was first showed brownish spots on the flesh with white mycelium (which was judged as "infected"). A longer storage period increased the development of white mycelium and powdery masses of green spores. Incorporated extracts in the wrapped films significantly reduced disease incidence in garlic, with the impacts of the treatment depending on the extract concentrations (Table 4). The incidence of disease on wounded inoculated garlic contacted to 2% crude extract or benomyl films was significantly lower than on garlic exposed to 0, 0.25, 0.5, or 1%.

At twelve days after storage, the wounded inoculated garlic exposed to treated gelatin films at the concentrations 0, 0.25, 0.5, 1, and 2 % had disease incidence of 100, 100, 86.67, 80, and 33.33%, respectively. The benomyl film also could not totally inhibit the fungal spore germination, with 6.67% of disease incidence on the 12th day of storage. The inability of 2% crude extract and fungicide to control the incidence of *A. flavus* may be attributed to the growth of fungal spores that growth inside the flesh of garlic tissues, which were not directly in contact with the tested films. The non-wounded inoculated group stored at the same temperature, on the other hand, they did not show any disease incidence throughout 12 days. The reason may be that *A. flavus* is a fungal pathogen that only attacks plants or tissues that have been damaged by various stresses, like artificial wounds in this study. They do not attack healthy tissues.

As a whole, the study showed the efficiency of gelatin films incorporated with 2% crude extracts from rain tree sawdust in retarding the growth of *A. flavus*, thus resulting in prolonged storage life of garlic. Gelatin film has been proven to increase the shelf life of fresh products by delaying the microbial spoilage and providing moisture and gas barrier properties (Said & Sarbon, 2022; Moradinezhad & Ranjbar, 2023). The addition of active ingredients into the gelatin films to maintain the quality and lengthen the shelf life of food, the films must have the ability to capture and release active ingredients into the products or the environment. Wang et al. (2017) added different concentrations of tannins to the gelatin solution by the casting method and their analysis showed a physical crosslinking effect between tannin and

gelatin, dominated by a hydrogen bond and hydrophobic bond. This finding proved that the incorporation of plant tannin into gelatin is practical. The hygroscopic nature of gelatin film allowed closed attachment between the film and the product's surface, active ingredients inside the film could be slowly released from the film to the moist surface of the products. Etxabide et al. (2022) incorporated grape tannin extracts into active gelatin films and discovered that the films may release tannins by up to 20%, resulting in antioxidant inhibition levels of up to 13%. According to Valdés et al. (2020), the active gelatin film's ability to limit microbial growth cannot be attributed to a single mechanism, but rather to a variety of reactions that take place on the entire microbial cell.

In the decomposition test of the film, at 36±2 °C and 41% RH, the 0% film treatment started to decompose within a week. The 0.25, 0.5, and 1% treatments were followed by a decomposition process that began within 2-3 weeks. Said and Sarbon (2022) stated that the biodegradation rate of single gelatin films was 18-25 % after 3 days. The higher moisture makes the films become more susceptible to degradation towards microorganism attacks. The incorporation of additional substances in the film matrix will lower moisture content, thus lowering the biodegradation rates. After 1 month of the degradation test, the stable structure of the film was found in the benomyl and the 2% treatment; both treatments started a little bit distorted but still degraded less than 25% (Fig. 1). As a result, the 2% film treatment could be naturally decomposed and had a lifetime of more than 1 month, and after that, it started to gradually deformation. In this sense, the treated films in this work can degrade naturally through biodegradation. The potential use of gelatin films as substitute materials for packaging applications is highlighted by their excellent functional qualities and favorable environmental impact (Etxabide et al., 2016).

Table 4. Percent of disease incidence on wounded inoculating garlic with *A. flavus* after being wrapped by gelatin films incorporated with various concentrations of crude extracts from rain tree sawdust or benomyl and then kept at 35±2°C for 12 days.

Treatment	Percent of disease incidence (%)
0%	100.00^{a}
benomyl	6.67^{d}
0.25%	100.00^{a}
0.50%	86.67^{ab}
1 %	80.00^{b}
2%	33.33^{c}

Different superscripts within the same column indicate statistically significant different values ($P \leq 0.05$).

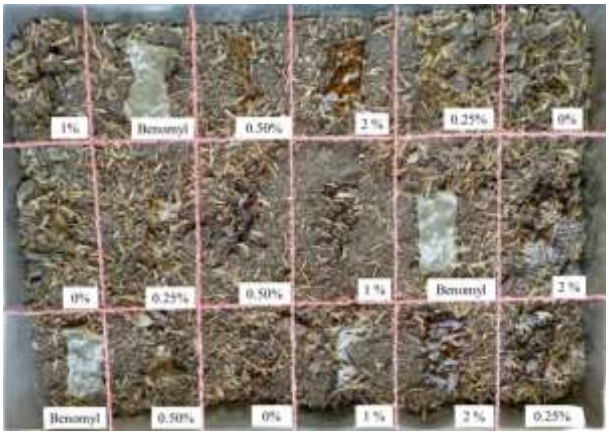

Fig. 1. The appearance of the gelatin films incorporated with various concentrations of crude extracts from rain tree sawdust, after 1 month of the degradation test at 35±2°C, 41% RH.
Note: The tested films with three replications were arranged randomly, each row had all treatments.

CONCLUSION

In conclusion, the optimum condition in extracting the sawdust from the raintree was 100 watts of the microwave with the mixed solvent of distilled water: 95% ethanol (1:1 v/v) which gave the highest yield of extract and tannin content. The extracts could inhibit mycelium growth and spore germination of *A. flavus*, especially at 2% of the concentration used. The incorporated 2% extracts into gelatin films also prolonged the storage life of wounded inoculating garlic with *A. flavus* for more than 12 days. All treated films in this study could be naturally gradual decomposed within a month.

Conflict of interest

The author has no conflict of interest to report.

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Aloe vera gel coating maintains physicochemical parameters, extends the storage life, and preserves the qualities of Lantundan and Cavendish bananas

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ABSTRACT

Purpose: Bananas have been experiencing increased production worldwide due to increased cultivated areas over the last three decades. However, postharvest losses of bananas are the major concern due to their highly perishable nature and therefore require appropriate treatments and storage methods to extend storage life. This study evaluates the effects of *Aloe vera* coating and storage temperatures on the physiological changes, and sensorial attributes of Lantundan and Cavendish bananas. **Research method:** These fruits were coated with *Aloe vera* and stored at 10°C or 25 ± 2°C and relative humidity of 70-75% or 50-55% respectively. The fruits were evaluated every 2 days for 10 days. **Findings:** *Aloe vera* treatment reduced weight loss, inhibited peel colour changes, delayed total soluble solids and titratable acidity changes, and minimised decay of the two banana cultivars. Coating did not significantly affect taste and overall acceptability, although panelists preferred coated bananas. The combined effects of *Aloe vera* coating and storage at 10°C was the most effective treatment in maintaining Lantundan and Cavendish bananas qualities. **Research limitations:** This study could not measure endogenous ethylene and respiration to ascertain their impact on peel colour changes to a lack of equipment**. Originality/Value:** It therefore extended the shelf life of the fruits to 10 days, compared with uncoated bananas which had a shorter shelf life of 6 days.

INTRODUCTION

Bananas (*Musa* spp) are a perennial crop that grows quickly and can be harvested all year round. Bananas are grown in tropical regions using sucker, and tissue culture and play a key role in the economies of many developing countries. Bananas are climacteric; they ripen rapidly and soften after harvest (Huang & Jiang, 2012), and have a short storage life (Gol & Rao, 2011), due to their susceptibility to decay caused by microorganisms (Deka et al., 2006). Most cultivars are yellow when ripe but some are purplish or red. About 1,000 banana cultivars have been identified in more than 150 countries (Li & Ge, 2017). Banana production increased from 97 million tonnes (Mt) in 2008 to about 120 Mt in 2020 from cultivating 5.2 million hectares (FAOSTAT, 2022), indicating an increase in production worldwide, but postharvest losses of bananas are about 40% in developing countries, such as Ghana (Al-Dairi et al., 2023). Bananas are ready for harvest when they are still green, and when ripening begins, they are subject to physiological and biochemical changes, hence synthesizing various volatile compounds that affect flavour and peel colour changes (Maduwanthi & Marapana, 2019). This influences ripening and browning therefore leading to critical quality problems, such as weight loss due to respiration and transpiration, softening of flesh, and lack of resistance capacity against microbial attack (Aziz et al., 2021). These physiological issues cause considerable economic losses to banana producers (Aziz et al., 2021). To solve this problem, treatments with edible coating before storage could be used to maintain the quality of banana fruits.

An edible coating is a thin layer coating on the surface of food through application, dipping, spraying, etc., to provide physical protection while selectively preventing the permeation of gases and escape of water from horticultural produce (Baldwin & Hagenmaier, 2011; Firdous et al., 2023). Edible coatings are biodegradable, environmentally friendly, and possess antifungal properties (Saks, 1995), hence have preservative effects (Kuorwel et al., 2015). The main materials for making edible coating usually originate from plants and animal products, especially *Aloe vera* gel (Petersen et al., 1999). Several studies have evaluated the effects of coatings on fresh agricultural produce (Supa et al., 2024). For instance, the applications of edible coatings have been shown to have provided a selective barrier to moisture loss and retained firmness of table grapes (Valverde et al., 2005), and gas and solute migrations, therefore extending the storage life (Aloui & Khwaldia, 2016). In addition, edible coating improved the sensual qualities of fruit, giving it a gloss and preventing colour change (Kim et al., 2022), maintaining textural quality, retaining volatile flavour compounds, and reducing microbial growth (Debeaufort et al., 1998). Moreover, reports showed that *Aloe vera* coating maintained firmness, delayed kiwifruit fruit yellowing, and ascorbic acid decline (Benítez et al., 2015), and decreased respiration rate, oxidative browning and the growth of microorganisms in table grapes (Valverde et al., 2005).

Edible coating and ambient storage reduced weight loss and extended Cavendish bananas' storage life (Dwivany et al., 2020). Bananas stored at low temperatures alone have less weight loss and deterioration compared with fruit kept at ambient conditions (Cano et al., 1997; Moradinezhad et al., 2008). The primary mechanism that contributes to fruit weight loss is vapour-phase diffusion driven by water vapour gradient between the inside and outside of the fruit leading to increase transpiration (Suseno et al., 2014). Recently, Bantayehu and Alemayehu (2020) indicated that holding bananas at 15°C was optimum for inhibiting peel colour changes relative to ≥20°C storage. Consumers often evaluate banana fruit quality mainly by colour, brightness, and size. These criteria are complemented by firmness, total soluble solids (TSS), and acidity (Moreno et al., 2021). A study reported that a decrease in TA of bananas due to a decrease in organic acid content, adversely affected internal quality

(Thakur et al., 2019). Other investigations observed that changes in sugar and acid ratios in bananas influenced the organoleptic taste and consumer acceptability during storage (Pott et al., 2020).

The application of edible coating is promising to improve the quality and extend the storage life of fruits since it can form a perfect coating on fruit surfaces to delay the ripening and retain quality properties, and it is regarded as a safe material. Banana storage using *Aloe vera* gel as the edible film is relatively a new treatment method. Currently, little study has applied this concept to a few banana cultivars as discussed in previous sections. Hence, the objective of this study was to evaluate the impact of *Aloe vera* coating and two storage temperatures (10°C or 25 ± 2°C) on physiological and biochemical changes in two banana cultivars (Cavendish and Lantundans) and to maintain desirable quality factors and extend their postharvest life.

MATERIALS AND METHODS

Aloe vera gel preparation

Aloe vera plant (*Aloe barbadensis*) was obtained from a farmer in the Wa municipality in the Upper West Region of Ghana. The plant was cleaned to remove dirt using napkins and the edges were cut. The central part of the leaf averaging 85% of total weight was the pulp used as the portion for the gel coating. To extract the gel, the pulp was cut into smaller pieces using a sharp knife, and this material was washed for a minute with tap water and left in an ambient for 5 hours to air dry off the residue water. Immediately after drying the water off, the pulp was homogenised with an electric blender and filtered to remove purities. One percentage of 2.5% citric acid and 2.5% ascorbic acid were then added to the gel solution obtained to stabilise its content to form an *Aloe vera* gel of 25% of the total extract, according to the method of Arrubla-Vélez et al. (2021).

Plant materials and experimental design

Green mature Lantundan and Cavendish bananas were obtained from a farmer who is into the production of bananas from Techiman-Koase at Bono Ahafo region of Ghana. During harvesting, the bunches were removed from the plant by cutting a notch in the pseudo stem. The fruits were packaged in baskets lined with fresh banana leaves and jute sacks to minimise damage. The fruits were transported from Techiman for 5 h to Dr. Hilla Limann Technical University Postharvest Laboratory in Wa for the experiment to start. The hands were sorted based on visual defects, uniformity of weight, and shape. The hands were randomly divided into different treatment groups. Each treatment group consisted of eight uniform hands (8 fingers into a hand). In total sixty-four (64) hands of uniform-sized bananas were used for the experiment for both cultivars.

The bananas were wiped dry and the entire sample was divided into two based on cultivar with each cultivar having 32 hands of eight (8) fingers. The cultivars were further divided into four groups. The bananas hands were then dipped for 2 min with 25% *Aloe vera* gel, while control fruit were dipped in tap water and stored as follows; uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). The cold storage temperature was 10°C and relative humidity (RH) of 70-75%, while the ambient storage temperature was 25 ± 2°C with a 50-55% RH for 10 days. Fruits of each cultivar were randomly divided into two equal lots after cleaning and sorting.

The first lot was then surface coated with aloe vera while the second lot was used as control fruit. After the treatments, the fruits were stored at 10°C or 25 ± 2°C for ten (10) days. The fruits were kept in corrugated cardboard boxes and stored under the respective temperatures. Fruit were evaluated after every two days for weight loss (%), peel colour changes, titratable acidity, total soluble solids, fruit firmness, and fruit decay incidence. Cold and ambient temperature storage was monitored daily using tinny-tag data loggers during the experiment to determine the RH.

Evaluation of weight loss

The weight of individual treatments was recorded once every 24-hour interval for 10 days. The physiological weight loss was calculated for the above interval and converted into percentages. The percentage weight losses were obtained by subtracting the initial weight (W1) from the final weight (W2) divided by the final weight multiplied by 100% (1), i.e.,

$$\text{Weight} = W1\text{-}W2/W1 \times 100\% \qquad (1)$$

Assessment of colour changes

The average peel colour of the sample fruits was determined using banana maturity stage standardized colour charts. The peel colour of individual fruit was scored according to Venkata-Subbaiah (2013) with little modification. Briefly, the peel colour of the bananas was scored 1 to 7 scale, where 1 = all green, 2 = still green with traces of yellow, 3 = more green than yellow, 4 = more yellow than green, 5 = green tip, 6 = full yellow, and 7 = yellow with brown spots. The least numerical values in these ranging assessments show the betterment of the fruit's quality during the storage regime.

Internal quality measurement

The fruit TSS was measured by using a refractometer (Atago Co., Tokyo, Japan). The banana flesh was prepared to form a homogenous sample by blending in a blender after the removal of the banana peels. The sample was well mixed and a few drops of the juice were put on the prism of the refractometer, and readings were taken by recording the figures on the meter according to AOAC (1994). The TA was determined by titrating 5 ml of juice of two (2) fruits each from each hand. with 0.1 N sodium hydroxide, using phenolphthalein as an indicator (Mazumdar & Majumder, 2003), and the data expressed as percentage citric acid.

Determination of decay incidence

The decay percentage of the stored bananas was assessed according to Gol and Rao (2011) with little modifications. Briefly, on the assessment days of the experiment, all the fruit visual inspections were conducted, and fruits with physiological and microbial decay were discarded in each sample, and the decay percent was calculated and recorded.

Evaluation of sensory qualities and consumer acceptability

A blind taste test was performed on the fruits on day zero and day 10 of the experiment to ascertain consumer acceptance. In this, twenty (20) panel members tasted the fruit samples on the last day of storage for the treated and control samples and scored their observation according to a four-point hedonic scale; 1 = excellent, 2 = very good, 3 = good: limited of marketability, and 4 = Poor: unsalable.

Statistical analysis

The experimental design was a 3 × 2 factorial design arranged in a Completely Randomised Design. Banana fruits were selected based on uniformity and freed from defects. The data on external quality and physicochemical factors were statistically analyzed using two-way Analysis of Variance (ANOVA) using SPSS version 19. P-values of < 0.05 were considered statistically significant for the evaluated parameters. The average means comparison was done using Fisher's least Significant Difference (FLSD).

RESULTS AND DISCUSSION

Effect of coatings on weight loss

Bananas are one of the horticultural produce that is highly perishable and therefore need effective treatments and storage conditions to enhance fruit-keeping qualities. The results obtained on weight loss of Cavendish and Lantundan bananas coated with *Aloe vera* and stored at 10°C for 10 days showed that Cavendish coated with aloe vera coatings on day 4 produced an average weight loss of 2.3% as compared with coated Lantundans of 0.9% as opposed to control fruit experiencing a weight loss of 4.6% and 3.9% respectively. On day 10, the results obtained from weight losses indicated that there was an increase in weight on coated Cavendish lost 2.7% compared to Lantundans with 2.1%, but the weight loss of control increased to 10.6% and 7.9% respectively (Fig. 1), which confirms the results of Suseno et al. (2014) where control bananas lost more weight compared with coated bananas. According to Quoc (2021), *Aloe vera* coating is capable of forming a natural gas barrier and extends banana storage life.

On day 4, the weight loss of coated Cavendish fruit held at 25 ± 2°C recorded an average weight loss of 1.8% compared with Lantundans of 1.5%, however, both their controls had a weight loss of 4.4%. However, on day 10, the weight loss of coated Cavendish increased to 5.3% compared to Lantundans with 4.4% relative to control fruit with a weight loss of 15.3% and 13.1% respectively, and prolonged shelf life up to 10 days. However edible coatings and storage at ambient reduced weight loss and extended Cavendish bananas' storage life to 13 days (Dwivany et al., 2020). The primary mechanism that contributes to the weight loss of fruits is vapour-phase diffusion driven by water vapour gradient between the inside and outside of the fruit leading to an increase in transpiration (Suseno et al., 2014).

Storage temperature and cultivar had an interacting effect on the weight loss of fruits. The results showed that Cavendish bananas lost less moisture (about 10%) than the Lantundans, which could be due to differences in plant genetics. However, uncoated fruit stored at 10°C had a lower weight loss (13.1%) relative to the weight loss (15.3%) of fruits stored at 25 ± 2°C. The results also showed a significant effect of cultivar and temperature levels on weight loss, as Lantundans lost more moisture relative to Cavendish throughout storage under all the treatment and storage conditions (Fig. 1). The lowest weight loss was observed in both Lantundan and Cavendish bananas coated with *Aloe vera* and held at cold storage, which agreed with the finding of Quoc (2021), who decreased banana fruit weight loss with *Aloe vera* coatings.

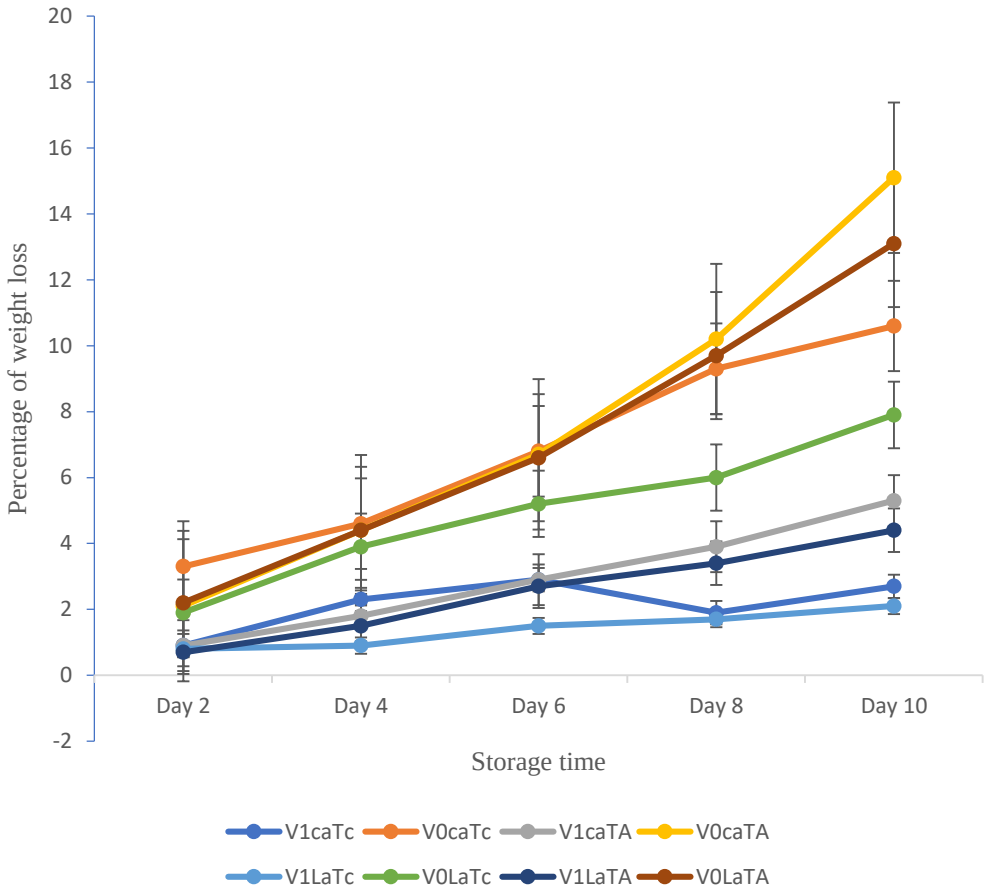

Fig. 1. Weight loss of coated and uncoated Lantundan and Cavendish bananas stored at cold and ambient temperatures. Uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). Each value is a mean of triplicate determination.

Peel colour changes

Banana peel colour is an important factor that influences consumers to buy from the market and therefore must be maintained. This investigation showed a significant ($p<0.05$) difference in the peel colour of coated bananas and control stored at cold and ambient conditions. Bananas coated with *Aloe vera* appeared to have less brown peel than the control. A study has demonstrated that uncoated bananas showed an unsatisfactory appearance after 4 days of storage when compared with coated bananas which had an acceptable appearance for up to a week of storage (Suseno et al., 2014). Peel colour changes of Cavendish and Lantundans coated with *Aloe vera* and stored at 10°C showed that, on day 4 coated Cavendish was 1.9 compared with 1.8 for coated Lantundan bananas, regarding a score of 1.0 on day 0 of our study. While the peel colour of control Cavendish and Lantundans were 2.0 and 1.9 respectively. On day 10, the results showed that coated Cavendish recorded 1.9 compared with Lantundans of 2.4, control Cavendish and Lantundans scored 1.9 and 1.8 respectively. Colour changes of Cavendish and Lantundans stored at 25 ± 2°C indicated on day 4 that, colour changes occurred with both Cavendish and Lantundans as compared with the peel colour on day 0, where the peel scored 1.0. Cavendish and Lantundans coated with *Aloe vera* produced an average colour of 2.8 and 2.3, while uncoated Cavendish and Lantundan had an average colour value of 5.5 and 2.8 respectively. On day 10 of storage, the results showed that

coated Cavendish and Lantundans had an average colour of 5.1 and 4.5 respectively, relative to control Cavendish (7.0) and Lantundans (5.8), which demonstrated a steady peel colour development (Fig. 2). This result affirms the findings by Aziz et al. (2021), who showed steady colour changes in bananas with chitosan coating treatment. A study attributed delayed bananas' colour changes to the reduction of chemical changes, such as chlorophyll breakdown (Aziz et al., 2021). Generally, the results of the present study demonstrated that fruit kept at 10°C was better than those held at 25 ± 2°C. Similarly, Bantayehu and Alemayehu (2020) demonstrated that keeping bananas at 15°C had a superior peel colour to those held at room temperature (≥20°C).

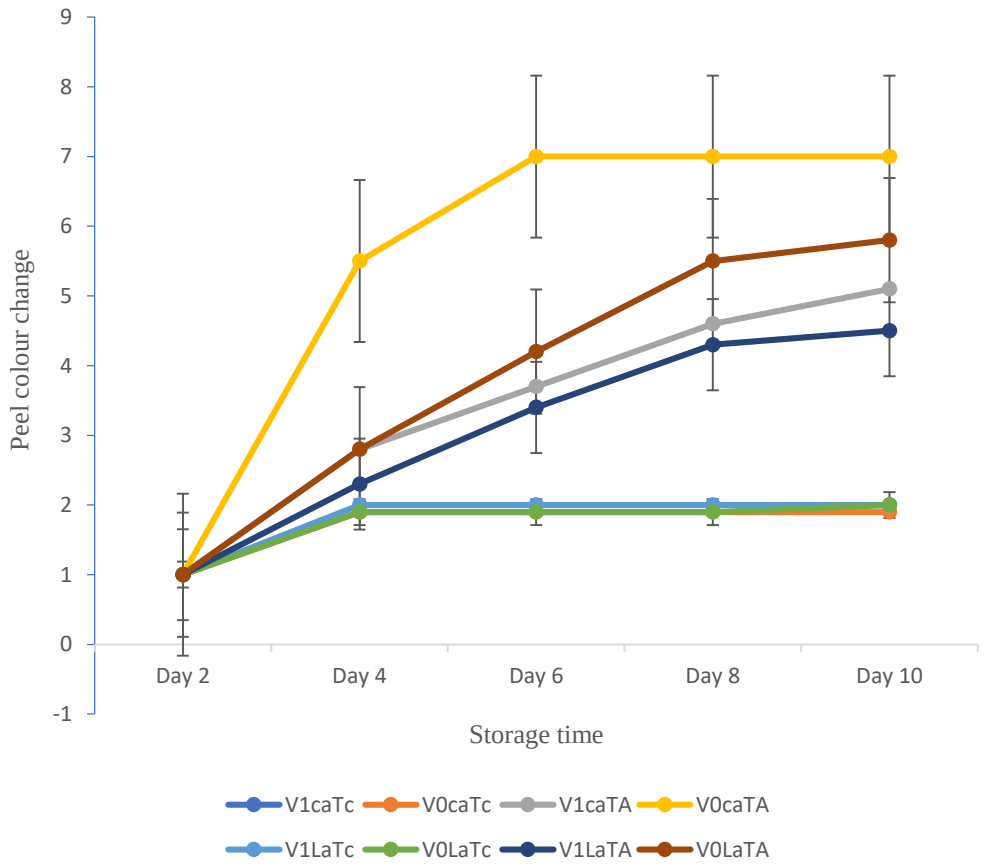

Fig. 2. Peel colour changes of coated and uncoated Lantundan and Cavendish bananas stored at cold and ambient temperatures. Uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). Each value is a mean of triplicate determination.

Total soluble solids and titratable acidity

There was a significant ($p<0.05$) effect of *Aloe vera* treatment and storage temperatures on the TSS of the fruits. Although, TSS usually shows a continuous increase with an increase in banana maturity (Wanna et al., 2001), in this study *Aloe vera* coating appeared to have significantly delayed a rise in TSS of Cavendish and Lantundan bananas. A comparison between the two cultivars in this investigation showed a similar trend, with Lantundans demonstrating a slight increase in TSS. Uncoated Lantundan held at 25 ± 2°C had a TSS of 18.6 °Brix relative to 23.4 °Brix for Cavendish stored at the same condition. TSS for coated Lantundan and Cavendish fruits showed an average of 15.1 and 19.2 °Brix respectively (Table 1). These differentials in TSS could not only be a result of levels of fruit maturity but rather could be due to plant genetics. On day 10, when the experiment was terminated, coated Cavendish bananas held at 10°C appeared attractive and with better eating quality. Coated bananas showed the lowest TSS values compared to uncoated bananas. Aloe vera, 0.6 and 1.2% for Arabic gum, and 100 and 200 g/l for starch are those that kept the TSS content of coated bananas low (Tchinda et al., 2023).

According to Thakur et al. (2019), the TA of bananas declined during storage as organic acid content decreased, and the reduction was ascribed to the conversion of organic acid into sugar. In this study, there was a significant effect of *Aloe vera* and storage temperature on TA of bananas. The average TA of control Lantundans was 0.37 on day 10, while that of coated Lantundans held at 10°C declined to 0.54, higher than an initial malic acid value of 1.02. However, coated Cavendish bananas held at 10°C showed the smallest decrease in TA compared with uncoated fruit kept at room temperature which showed a significant reduction of 0.63 from an initial 1.14. A cursory study of the decline in TA presented in Table 1, showed an interactive effect of treatments and storage conditions as well as in the two banana cultivars. In general, TA in this study showed a both banana cultivars decreased irrespective of whether it is coated. Still, the decline was more pronounced in uncoated Lantundans at room temperature. A slight decrease in the TA observed in coated Cavendish or Lantundans was observed, which was probably a result of the enzyme activity (Quoc, 2021). Moreover, Gol and Rao (2011) slowed the changes in TA by effectively delaying fruit senescence using chitosan coatings.

Table 1. Total soluble solids and titratable acidity of coated and uncoated bananas during cold and ambient storage.

Parameter	Treatment and storage conditions								
	Temperature	V0LaTA	V0LaTc	V1LaTA	V1LaTc	V0CaTA	V0CaTc	V1CaTA	V1CaTc
Banana spp		Lantundan bananas				Cavendish bananas			
TSS (°Brix)	Day 0	7.1				8.6			
	10°C	15.7^{b}	15.2^{b}	15.6^{b}	15.1^{b}	21.8a	20.3a	21.5a	19.2^{b}
	25°C	18.6^{d}	17.7^{d}	18.1^{d}	18.3^{d}	25.4^{c}	24.1^{c}	25.2^{c}	23.4^{c}
LSD (0.05)					2.6				2.6
TA (malic acid)	Day 0	1.02				1.14			
	10°C	0.54^{d}	0.69^{c}	0.66^{c}	0.75^{b}	0.67c	0.64c	0.72b	0.81^{a}
	25°C	0.37^{i}	0.51^{g}	0.54^{g}	0.58^{f}	0.52^{f}	0.46^{h}	0.57^{f}	0.73^{e}
LSD (0.05)					0.08				0.08

Uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). Values in the same column and rows with the same letters are not significantly different.

Table 2. Taste and flavour of coated and uncoated bananas during cold and ambient storage.

Parameter	Treatment and storage conditions								
	Temperature	V0LaTA	V0LaTc	V1LaTA	V1LaTc	V0CaTA	V0CaTc	V1CaTA	V1CaTc
Banana spp		Lantundan bananas				Cavendish bananas			
	Taste	2.2^{f}	2.1^{g}	2.4^{f}	2.5^{e}	2.6^{d}	3.4^{b}	3.2^{c}	3.8^{a}
	Flavour acceptability	2.6^{a}	3.4^{c}	3.0^{b}	3.7^{d}	4.1^{f}	3.8^{e}	3.5^{c}	4.2^{g}
LSD (0.05)					0.9				0.9

Uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). Values in the same column and rows with the same letters are not significantly different.

Sensory qualities and consumer acceptability

Fresh eating quality of bananas is an important parameter for consumer satisfaction and should be maintained after harvest. The results obtained from the taste test on fresh bananas scored by 20 panelists indicated that coated Cavendish and Lantundan bananas stored under 10°C recorded an average value of 3.8 and 2.5 and their control recorded 3.2 and 2.4 respectively, while uncoated Cavendish and Lantundans stored under ambient recorded 3.4 and 2.1 compared with 2.6 and 2.4 for uncoated bananas. Meanwhile, the overall acceptance for Cavendish and Lantundan bananas coated and stored under 10°C obtained 4.2 and 3.7 as compared with the control of 3.4 and 3.0 averages. The overall acceptance for control observed under ambient was recorded at 3.8 and 3.4 for coated Cavendish and Lantundans respectively, and uncoated samples held at room temperature rated as 4.1 and 2.6 for Cavendish and Lantundans respectively (Table 2). Generally, samples coated and kept at cold storage were rated higher than control but did not have significant effects on taste. However, there was a significant preference of tasters for Cavendish bananas relative to Lantundans. This is a demonstration that *Aloe vera* and storage temperature did not significantly improve bananas' sensory attributes. This effect contrasts the finding by Choi et al. (2016) who established that coatings had a greater impact on fruits and therefore provided a better flavour than control fruit.

Aloe vera coating had no significant effect on consumer acceptance due to coating and storage temperatures. However, storage temperatures and coating affected the bananas' taste. The highest acceptability score was observed in fruit coated with *Aloe vera* and stored at 10°C. Pott et al. (2020), observed that fruit taste and acceptability depend on the ratio of sugars and acids, which therefore determine flavour acceptability. However, treatment with *Aloe vera* maintained the peel colour of bananas in this study, especially those stored in a cold room. Both coated and control held at ambient conditions was off-flavoured, but this was more pronounced with uncoated fruit. Fruit with small colour changes due to *Aloe vera* coating did not also show off-flavour, hence had greater consumer acceptance. This positive effect of *Aloe vera* in this study is consistent with the findings of Valverde et al. (2005), who preserved the eating quality of sweet cherry with *Aloe vera* application.

Table 3. Decay incidence percent of coated and uncoated bananas during cold and ambient storage.

Parameter	Treatment and storage conditions								
	Temperature	V0LaTA	V0LaTc	V1LaTA	V1LaTc	V0CaTA	V0CaTc	V1CaTA	V1CaTc
Banana spp		Lantundan bananas				Cavendish bananas			
Decay incidence	Day 0	0							
	10°C	25.3^{a}	15.0^{c}	20.0^{b}	12.1^{d}	15.0^{c}	8.0^{e}	10.0^{d}	5.1^{f}
	25°C	35.0^{f}	20.0^{b}	30.3^{e}	26.0^{b}	23.2^{d}	18.0^{b}	21.0^{c}	15.1^{a}
LSD (0.05)					2.4				2.4

Uncoated Lantundans stored at ambient (V0LaTA); vera coated Lantundans stored at ambient (V1LaTA); uncoated Lantundans at cold storage (V0LaTc); vera coated Lantundans at cold storage (V1LaTc); uncoated Cavendish stored at ambient (V0CaTA); vera coated Cavendish stored at ambient (V1CaTA); uncoated Cavendish at cold storage (V0CaTc), or vera coated Cavendish at cold storage (V1CaTc). Values in the same column and rows with the same letters are not significantly different.

Decay incidence

Aloe vera coating in combination with cold storage significantly ($p<0.05$) inhibited the decay of the bananas as compared with the control. The decay of control was about 3 times higher than fruits coated with *Aloe vera* at the end of storage. Coated bananas stored at low temperatures had less decay (5%) for Cavendish relative to 12% for Lantundan bananas (Table 3). The effects of *Aloe vera* coating in reducing decay could be attributed to antifungal properties. Previous studies showed that chitosan acted as an antifungal property against postharvest pathogens (Jiang & Li, 2001). Moreover, a study reported that chitosan induces chitinase, a defense enzyme, and catalyzes the hydrolysis of chitin, which are common component of fungal cell walls, hence preventing the growth of fungi on the oranges (El-Ghaouth et al., 1992). This study shows an increased decay of both banana cultivars held at ambient, with uncoated fruit having a significantly higher decay than coated ones. This effect affirms the finding by Aziz et al. (2021), who observed the lowest disease level in chitosan-coated bananas.

There was a significant difference in the decay of bananas during the storage regime. A decay of 35% was observed for the Lantundans held under ambient conditions compared with a loss of 25% for Cavendish fruit at the same storage conditions after 10 days. These results seem to suggest that Lantundan bananas are possibly more susceptible to decay than Cavendish bananas and hence had a higher decay percentage. Bananas are highly perishable due to physiological alterations such as weight loss, as a consequence of respiration and transpiration, fruit softening as well and less resistance capacity against microbial attack. Higher respiration rate for example is mostly a possible factor for higher decay. Hailu et al. (2014) noticed that an increase in respiration led to tissue softening of bananas, which in turn increased the level of rot during storage. Similarly, Aziz et al. (2021), reported that delaying the physiological process and loss of weight could inhibit microbiological activity and possibly prolong bananas' storage life.

CONCLUSION

The present study reveals that the combination of *Aloe vera* coating and storage at 10°C was the best treatment for maintaining the quality and extending the shelf-life of Lantundan and Cavendish banana cultivars. After a storage period of 10 days at 10°C and 25 ± 2°C, the results indicated that *Aloe vera* gel can be an effective coating material to perverse physicochemical and eating quality and inhibit banana fruit rot. It was shown that the lightness and yellowness of the coated bananas were delayed with small losses at the end of storage while uncoated samples had unacceptable appearance and taste. Moreover, *Aloe vera*

coatings reduced moisture losses and colour changes, hence improving the bananas' quality. Based on these results, it can be safely concluded that the combination of *Aloe vera* coating and storage at 10°C was the most effective method in maintaining the quality and extending the shelf-life of both Lantundan and Cavendish bananas.

Conflict of interest

The author declares no conflict of interest regarding the publication of this work.

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