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The effects of cinnamic acid and IBA treatments on the rooting of wood cuttings of black mulberry (*Morus nigra* L.)†

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† This study is derived from a part of Nur Selin Karabulut's master thesis.

ABSTRACT

In this study, the effects of IBA and cinnamic acid applications on rooting of black mulberry cuttings taken from black mulberry trees grown in Tokat were treated. 6000 ppm Indole Butyric Acid (IBA), 6000 ppm IBA+100 ppm cinnamic acid (CA), 100 ppm cinnamic acid with control which no application was applied on the cuttings taken in July, September, November and January periods. The cuttings were kept during 60 days in rooting beds containing perlite and heated from the bottom. The highest rooting ratio (48.3%) and the highest average root number (3.1) were obtained from the cuttings taken November and January periods with 6000 ppm IBA + 100 ppm CA application. Among the applications and the periods, cuttings were taken, 6000 ppm IBA + 100 ppm CA application and July and January periods were superior to others.

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1. Introduction

Mulberry (*Morus* spp.) is the tree that creates the *Morus* genus of the *Moraceae* family of *Urticales* team, which can be grown in temperate, tropical and subtropical climates in the northern hemisphere by adapting to different climatic and soil conditions with high adaptability. The mulberry plant is common in many parts of the world (Datta, 2002). *Morus alba*'s homeland is China, Japan, Thailand, Malaysia and Burma; *Morus nigra* homeland is Turkey, Persia, Arabia, parts of Russia located in South Asia, and Syria; *Morus rubra*'s homeland is North America (Bellini et al., 2000). The species commonly made growing in Turkey; *Morus alba* (white mulberry), *Morus nigra* (black mulberry) and *Morus rubra* (red or purple mulberry). Turkey is one of the important producers with approximately 70000 tons of mulberry production (TUIK, 2020). In every region of Turkey which made the growing of fruit trees, mulberry cultivation can be done. Mulberry mostly likes warm, temperate and sunny regions. Optimum temperature demand of Mulberry is 24-28 °C (Özgen, 2010). It can be cultivated in places with annual rainfall amount is from 600 mm to 2500 mm and in places higher than sea level. The ideal humidity rate in mulberry cultivation is around 65-80% (Anonymous, 2013). Mulberry tree grows best in loamy, sandy-loam or clay-loam soils. The pH value of the soil should be 6.5-7. Groundwater should not be close to the soil surface, especially where the mulberry tree is planted. Mulberry is not selective in terms of soil and climate conditions except salty soils (Özgen, 2010). In mulberry production, vegetative methods such as grafting, layering tissue culture and production with cutting are used. In process of propagation by grafting, because of its high labor force and the lack of educated people to graft limits the success of grafting. In production with tissue culture, the desired level could not be reached in practical terms due to the need for special techniques and equipment. Due to the better results obtained compared to other vegetative reproduction methods, the most used method is the propagation by cutting (Anonymous, 2013). Propagation by cutting varies depending on the time of taking the cutting, the species and variety, the type of cutting, and the ecological conditions of the region where the main plant is grown. In the cutting reproduction method, generally, perlite is used in rooting environments and IBA hormone is used to increase rooting (Ryu, 1977). Many researchers working on the reproduction of mulberry with cuttings determined that rooting-stimulating hormones increase the root formation in mulberry cuttings (Şenel, 2002). In the cutting reproduction of mulberry plant, generally commercially available IBA's concentrations of 4000-8000 ppm are used. Soft cuttings are immersed in the prepared solution for 5-10 seconds and planted in the rooting medium. To achieve more successful root development, especially in rooting of wood cuttings, to heat the root regions at 21-27 °C during the day, and at 16-21 °C during the night will increase rooting by promoting cell division (Anonymous, 2013). Ünal et al. (1992) stated in their study that it did not reach maximum rooting rates with also IBA doses in black mulberry. In the studies to determine the rooting performance of black mulberry cuttings, rooting rates of up to 60% were reached by using different IBA concentrations (2000-6000 ppm) (Karadeniz and Şişman, 2003; Koyuncu and Vural, 2003; Erdoğan and Aygün, 2006). In many studies, it has been stated that the sugar and protein content, as well as the change in phenolic compounds within the plant, plays an important role in terms of adventive root formation (Kaur et al., 2002; Qaddoury and Amssa, 2004;

Kevresan et al., 2007; Satish et al., 2008). Researchers have stated that some phenolic compounds promote rooting while others prevent (Schneider and Wightman, 1974; Faust, 1989; Bandurski et al., 1995). It has been determined that especially when cinnamic acid is used together with IBA, it has a positive effect in terms of rooting performance (Padney and Pathak, 1981).

This study aimed to investigate the effects of cinnamic acid, IBA, and period differences on rooting in black mulberry cuttings that are difficult to root.

2. Materials and methods

The research was carried out in the rooting greenhouse of Tokat Gaziosmanpaşa University Agricultural Application and Research Center in 2014-2015. The research was planned in four different periods with four different applications and three repetitions. In the study, cuttings taken from old branches were prepared in 15 cm length and 15 cuttings were planted in each repetition. Cuttings were put in a heated mist propagation unit which was filled with perlite. Keeping the rooting temperature and humidity values at the desired level were achieved by a controlled automatic system. Before planting, the cuttings were immersed in a fungicide solution to protect against fungal infections. While preparing the solutions, the active ingredients were first dissolved in methanol and then water and methanol were added to the mixture according to the dilution ratio to be used. 1 cm of the cuttings from the bottom was immersed in 6000 ppm IBA, 100 ppm CA and 100 ppm CA + 6000 ppm IBA solutions for 5 s. Then, the cuttings were kept for 1-2 min and the alcohol on them was allowed to evaporate. Since the period differences are considered, planting was done in 4 periods. Cuttings planted on tables were kept in a rooting medium for 2-3 months. At the end of this period, measurements were made on the cuttings removed from the rooting medium.

Callus rate (%): Callus cuttings were counted and expressed in % (Edizer et al., 2016).

Rooting rate (%): Rooted cuttings were counted and expressed in % (Edizer et al., 2016).

Average number of roots per plant (number/plant): All roots of cuttings rooted in each plot were counted and recorded and the average of the results was calculated (Edizer et al., 2016).

Average root length (mm): The average of the results was calculated in mm by measuring with a digital caliper the roots of the cuttings rooted in each plot (Saracoglu et al., 2016).

Root diameter (mm): The diameters of the cuttings rooted in each parcel were measured with a digital caliper and the results were calculated in mm (Saracoglu et al., 2016).

The study was arranged according to the random parcel trial pattern in three repetitions. Fifteen cuttings were used in each repetition. Obtained data were subjected to analysis of variance (ANOVA) by using SAS software, and LSD test was preferred for comparing the averages.

3. Results and discussion

As it can be understood from the Table 1 it was determined that the effect of plant growth regulator doses on black mulberry wood cuttings callus rate was statistically significant at 5% level. The highest callus rate was obtained from 6000 ppm IBA dose in January (97.8 %). The lowest callus rate was obtained from 100 ppm CA dose in November (2.2%). In terms of average callus rate treatments 6000 ppm IBA had the highest callus rate. It was found that the callus rate was highest in July, while the lowest callus rate in November. In the study of Özkan et al. (1995), it was stated that the best callus formation occurred in green cuttings with 6000 ppm IBA application.

Table 1. The effect of plant growth regulator concentrations applied on callus rate (%)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	88.9 A-a	82.2 A-a	2.2 B-b	68.9 A-a	60.6 a
100 ppm CA + 6000 ppm IBA	93.3 AB-a	64.4 B-a	31.1 C-ab	95.6 A-a	71.1 a
6000 ppm IBA	86.7 A-a	68.9 A-a	35.6 B-a	97.8 A-a	72.2 a
Control	89.0 A-a	75.6 A-a	24.4 B-ab	93.3 A-a	70.6 a
Average	89.47 A	72.8 B	23.3 C	88.9 A	68.6

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important ($P<0.05$).

When Table 2 is examined, the highest rooting rate in black mulberry wood cuttings dose of 100 ppm CA+6000 ppm IBA in July (%88.9), while the lowest rate of rooting at the dose of 100 ppm CA in November (%0.0). In terms of average rooting rate, treatment of 100 ppm CA+6000 ppm IBA has highest rooting rate. In the black mulberry wood cutting the highest rooting rate in January, while in September the lowest rooting rate. In the study conducted by Kako (2012) the lowest rooting rate was determined in the control groups. Similarly, Mohammed and Kako (2021) stated that high IBA doses were effective in increasing the rooting rate.

As can be seen from Table 3 dose of 100 ppm CA+6000 ppm has the highest average number of root in July (5.7), the lowest average number of root 100 ppm CA dose in November (0.0). In terms of doses applied in black mulberry cuttings average the number of root was highest in 100 ppm+6000 ppm IBA dose, followed by 6000 ppm IBA and 100 ppm CA doses. It was found that the average number of root was highest in July, while the lowest average number of root in November.

Table 2. The effect of plant growth regulator concentrations applied on the rooting rate (%)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	31.1 B-b	11.4 BC-a	0.0 C-b	60.0 A-a	25.6 b
100 ppm CA + 6000 ppm IBA	88.9 A-a	8.9 D-a	31.1 C-a	64.4 B-a	48.3 a
6000 ppm IBA	83.0 A-a	6.6 C-a	22.2 C-a	55.5 B-a	41.8 a
Control	22.3 B-b	6.7 B-a	24.4 B-a	46.7 A-a	25.0 b
Average	56.3 A	8.4 C	19.4 B	56.7 A	35.2

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important (P<0.05).

Yıldız et al. (2009) found that the highest number of roots with 6000 ppm IBA application in the wood cuttings taken in winter and green cuttings taken in the summer period. Similarly, Edizer et al. (2016) stated that high IBA doses were effective in increasing the number of roots. As it can be understood from the Table 4 it was determined that the effect of plant growth regulator doses on black mulberry wood cuttings root length was statistically significant at 5% level. The highest root length was obtained from 100 ppm CA + 6000 ppm IBA dose in July (109.1 mm). The lowest root length was obtained from 100 ppm CA dose in November (0.0 mm). In terms of root length treatments 100 ppm CA+ 6000 ppm IBA had the highest root length. It was found that the root length was highest in July, while the lowest root length in September. Many researchers found that the highest average root length with 6000 ppm IBA application in the wood cuttings taken in winter and green cuttings taken in the summer period (Yıldız et al., 2009; Cekic et al., 2013).

Table 3. The effect of plant growth regulator concentrations applied on the average root number

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	1.6 A-b	2.5 A-a	0.0 A-a	3.2 A-a	1.8 a
100 ppm CA + 6000 ppm IBA	5.7 A-a	2.7 AB-a	1.7 B-a	2.3 B-a	3.1 a
6000 ppm IBA	4.6 A-ab	3.0 A-a	1.6 A-a	2.8 A-a	3.0 a
Control	2.3 A-b	2.2 A-a	1.5 A-a	2.1 A-a	2.0 a
Average	3.6 A	2.6 AB	1.20 B	2.6 AB	2.6

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important. (P<0.05).

As stated in Table 5 doses of 100 ppm CA and 6000 ppm IBA have widest root diameter in July (1.9 mm), while dose of 100 ppm CA has narrowest root diameter in November (0.0 mm). In terms of root diameter treatments 100 ppm CA have widest root. It was found that root diameter was widest in July, while the narrowest root diameter in September. Yıldız et al. (2009) found that the highest root diameter ratio with 6000 ppm IBA application in wood cuttings taken in the winter period, green cuttings are taken in summer period and semi-wood cuttings taken in October. In addition, many researchers stated that the lowest root diameter values were obtained from the low IBA doses samples (Cekic et al., 2013; Singh, 2018).

Table 4. Effect of applied plant growth regulator concentrations on mean root length (mm)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	68.3 A-a	50.4 A-a	-	84.9 A-a	67.9 a
100 ppm CA + 6000 ppm IBA	109.1 A-a	37.8 B-a	50.9 B-a	89.5 AB-a	71.8 a
6000 ppm IBA	86.9 A-a	24.1 B-a	44.7 AB-a	50.9 AB-a	51.6 a
Control	88.9 A-a	24.3 B-a	46.0 AB-a	49.5 AB-a	52.2 a
Average	88.3 A	34.1 B	47.2 B	68.7 A	60.9

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important. (P<0.05).

4. Conclusion

In our study, it was investigated the changes in the rooting performance of the cuttings as a result of using indole butyric acid and individually and together cinnamic acid in different cuttings taking periods in black mulberry plant. According to the findings, the best rooting rate was found as 88.9% in the cuttings treated with 6000 ppm IBA + 100 ppm CA in July. The best mean root number was 5.7 in the cuttings treated with 6000 ppm IBA + 100 ppm CA in July and the highest root length was 109.1 mm in the cuttings treated with 6000 ppm IBA + 100 ppm CA. 100 ppm CA + 6000 ppm IBA treatment was generally more successful and it was determined that the periods in January and July were the most successful. The most successful treatment terms of rooting rate, which is of great importance for rooting performance, was found as 100 ppm CA + 6000 ppm IBA treatment in July. According to the results of this study, we

can say that 6000 ppm IBA + 100 ppm CA treatment on the cuttings taken in July and January is the most suitable growth regulator dose for rooting, root number, root length and root diameter in rooting wood cuttings of black mulberry variety in Tokat region.

Table 5. The effect of applied plant growth regulator on average root diameter (mm)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	1.9 A-a	1.0 AB-a	-	1.6 A-a	1.5 a
100 ppm CA + 6000 ppm IBA	1.8 A-a	0.4 B-a	0.7 AB-a	1.0 AB-a	0.9 a
6000 ppm IBA	1.9 A-a	0.6 B-a	1.0 AB-a	0.8 AB-a	1.1 a
Control	1.4 A-a	0.9 A-a	0.9 A-a	1.3 A-a	1.1 a
Average	1.8 A	0.7 B	0.9 B	1.2 B	1.2

The differences between the period averages shown in the same capital letter in the same row and the application averages sho wn in the same column in the same lower case are not important. (P<0.05).

This study, in which we investigated the effect of cinnamic acid and indole butyric acid treatments on rooting in black mulberry cuttings, is important in terms of providing a source for hormone applications in future studies of reproduction with cutting. Studies to be done on this subject in more detail, to development of mulberry cultivation and sapling production in Turkey will be beneficial. When the findings we obtained were examined, the highest values in callus formation, rooting rate, average root number, average root length, and root diameter were reached in 6000 ppm IBA and 100 ppm CA + 6000 ppm IBA applications. Considering the period, the best results were obtained in July.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Nur Selin Karabulut: participated in data collection, analysis, description, and draft the manuscript. **Onur Saraçoğlu**: participated in supervision of the work starting from the proposal up to final draft, edited and revised the manuscript.

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The status and determinants of rural household food insecurity in North Shewa Zone, Oromia Region, Ethiopia

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ABSTRACT

Food insecurity and undernutrition are significant challenges to the economic growth of Ethiopia. The objectives of this study were to identify the status and determinants of food insecurity for households targeted under a productive safety net program. About 392 beneficiary households were selected using multistage random sampling methods. The household food balance model and multiple linear regression models were used to measure household food insecurity levels and identify factors affecting food insecurity. According to the food balance model output, all sample households targeted under the program were food insecure. This revealed that the total daily energy available to the sample households was less than 2100kcal per adult equivalent. Moreover, the multiple regression model output depicts that family size, sex of the respondents, total farm owned by the respondents, livestock size, credit utilization, household participation in community organization, household access to off/non-farm income, and household access to saving habit had a significant effect on food insecurity status of households. Therefore, in order to reduce the food insecurity status of households policymakers should focus on strengthening household saving habits, expanding off/non-farm activities, promoting family planning, and strengthening the credit services.

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1. Introduction

Food security is a state or a condition in which people experience unlimited physical and economic access to safe, sufficient, and nutritious food to meet their dietary needs or food preferences for a productive, healthy and active life (FAO, 2000). According to the World Bank/Ethiopia (2015) report, Ethiopia is one of Africa's fastest growing populations and economies. Despite increasing investment in agricultural extension work and input utilization, agricultural productivity remains low by international standards due to land degradation, drought, lack of irrigation, and constraints in input utilization (WFP and CSA, 2019). This brings Ethiopia to face high levels of food insecurity, ranking as one of the hungriest countries in the world (Global Hunger Index, 2009). Although the level and intensity vary, food insecurity exists in many parts of developing countries (Degefa, 2005). The cause of food insecurity varies depending on factors affecting the four pillars of food security (food availability, food access, food utilization and food stability). Limited resource and increased food price affecting many households of the world including Ethiopia are the common factors that affect food insecurity (Carter et al., 2010; Belachew et al., 2012).

To decrease the prevalent chronic food insecurity, the government of Ethiopia launched the most extensive rural social protection program called Productive Safety Net Program (PSNP) aimed to fill the household food gap, increase community assets, mitigate shocks such as drought, and ultimately attain food security (Devereux et al., 2006; MoARD, 2014). The program is planned to be implemented for five years, at the end of which PSNP beneficiaries who have received predictable transfers and complementary interventions throughout the program period will be expected to graduate out of dependence on external support, except during food crises (Arega, 2012).

Among the 13 districts found in North Shewa Zone, 5 districts namely Abichugnea, Wuchale, Jidda, Kimbibit, and Kuyu are the beneficiary of productive safety net program (North Shewa Zone Agriculture and Natural Resource Office, 2020). However, majority of beneficiary households targeted under the program are not food self-sufficient. Therefore, this study measures the status and determinants of households' food insecurity in the study area. To achieve these objectives primary data was collected from 392 beneficiary households participated in public work during the 2019/20 production year.

2. Materials and methods

2.1. Sampling techniques and sample size determination

Purposive and multistage random sampling techniques were used to select the beneficiary households in the study area. In the first stage, among 13 district existed in North Shewa Zone, 5 districts were purposively selected based on the districts access to safety net program. In the second stage, 10 sample *kebeles* (smallest administrative unit in the Ethiopian government structure) were purposively selected from each district based on the *kebeles* access to safety program. In the third stage, beneficiary households in each sample *kebeles* were proportionally distributed and randomly selected for interview (Table 1). The total sample size is calculated by using the simplified Yamane (1967) formula specified in equation 1.

$$n = \frac{N}{1 + N(e)^2} = \frac{19{,}635}{1 + 19{,}635(0.05)^2} = 392 \quad (1)$$

Where;

n = Sample size
N= Total beneficiaries households participated in public work in the sample district (at least for five years)
e = margin of error (0.05)

Table 1. Sample size determination procedures

Name of districts	Name of kebeles	Total households	Sample households
1. Kuyu	Jila Qerensa	91	13
	Tamsasa Roge	24	4
2. Abichugnea	Ano Akabdo	137	20
	Doyo Dawe	166	24
3. Wuchale	Nono	178	26
	Walanso Aroji	119	17
4. Jidda	Gango	894	131
	Wanya Daga Nasri	681	100
5. Kimbibit	Daka Bora	187	27
	Gara Catu	205	30
Total		2,682	392

Source: NSZANRO data (2020)

2.2. Data collection techniques

The primary data was collected through a survey and key informant's interview. To generate quantitative and qualitative data at the household level, a household survey was undertaken by developing questionnaires. Before full implementation, questionnaires were pre-tested, and necessary adjustments are made. To supplement primary data, a key informant interview was also conducted.

2.3. Methods of data analysis

Descriptive statistics like frequencies, percentage, mean and standard deviation were used to analyze data. Both SPSS (statistical package for social science) software and Microsoft excel was used to generate the output. Following Degefa's (1996) household food balance model specified in equation 2 was used to estimate the food insecurity level of sample farmers.

$$NGA = (GP + GB + FA + GG) - (HL + GU + GS + GV) \quad (2)$$

Where;

NGA = Net grain available to farmers in one year
GP = Total grain produced by sample farmer in one year
GB = Total grain bought by sample farmer in one year
FA = Amount of food aid obtained by a farmer in one year
GG = Total grain acquired as a gift or remittance by farmers in one year
HL = Post-harvest losses in a given year
GU = Amount of grain kept for seed by farmers in one year
GS = Amount of grain sold by a farmer in one year
GV = Amount of grain given to others in one year

The threshold for categorizing households into food secure and food insecure is 2100 kcal/day/adult equivalent (AE). Accordingly, sample households with daily caloric consumption of less than 2100 kcal/day/AE were considered food insecure while greater than or equal to 2100 kcal/day/AE were food secure (Kaluski et al., 2002). The household food balance assessment covers a period between September 2019 and September 2020. In this model, the data used for the computation is generated through field survey except the estimates given for the Amount of grain reserved for seed and post-harvest loss. Following Mesay (2001), the estimate for total grain kept for seed, and post-harvest loss was 5% and 10% of complete grain produced by sample households, respectively.

Previous researchers used the binary logistic regression model to estimate the determinants of food security (Ayalneh and Shimelis, 2009; Achenef et al., 2016; Moroda et al., 2018) due to the binary nature of a dependent variable. However, this study used multiple linear regression models to analyze the determinants of rural households' food insecurity because the dependent variable is continuous (total amount of energy consumed by households per day). Following Gujarati (1995), the model is specified as follows:

$$Y_i = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \beta_8 x_8 + \beta_9 x_9 + \beta_{10} x_{10} + \beta_{11} x_{11} + \beta_{12} x_{12} + \beta_{13} x_{13} + \varepsilon \quad (3)$$

Where; Yi= Amount of energy available to household per adult equivalent per day
β_o= Intercept
β_1 *to* β_{13}= Vectors of parameter to be estimated
ε= Error term
X_1 to X_{13} = Vectors of explanatory variables included in the model as listed below:
X_1= Family size

X_2= Sex of household head
X_3= Farm size
X_4= Livestock size
X_5=Access to credit
X_6= Participation in community organization
X_7= Access to off/non-farm income
X_8= Access to saving
X_9= Soil fertility
X_{10}= Access to extension
X_{11}= Education level of household head
X_{12}= Age of household head
X_{13}= Distance from market

2.4. Definition, measurement, and hypothesis of the study variables

Dependent variables: It is a continuous variable and defined as the total amount of energy consumed by household per AE per day.

Independent variables: It is a dummy and/or continuous variable and defined as the vectors of explanatory variables hypothesized to affect rural households' food insecurity status in the study area (Table 2).

Table 2. Summary of explanatory variables included in the model

Variables Name	Types of variables	Expected effect
1. Age of household head	Continuous	+
2. Sex of household head	Dummy	+
3. Education level of household head	Continuous	+
4. Family size	Continuous	-
5. Livestock size	Continuous	+
6. Access to credit services	Dummy	+
7. Participation in off/non-farm activities	Dummy	+
8. Access to extension services	Dummy	+
9. Farm size	Continuous	+
10. Soil fertility	Dummy	+
11. Distance from market	Continuous	-
12. Access to off/non-farm income	Dummy	+
13. Participation in community organization	Dummy	+

Source: Own construction based on literature (2021)

3. Results and discussion

3.1. Socioeconomic and farm characteristics of the respondents

Education level of household head: Education is the key to improve agricultural production, productivity and food security level of household. The survey result show that, the average education level of household head was 0.59 grades ranging from 0 to 4 grades implying that sample households in the study area did not attend more education (Table 3).

Table 3. Socioeconomic and farm characteristics of the respondents

Variable name	Mean	Std. dev.	Min	Max
Education level of household head	0.59	0.17	0.00	4.00
Family size	3.94	0.76	1.75	6.00
Age of household head	54.56	10.93	28.00	87.00
Farm size	0.91	0.33	0.50	3.00
Livestock size	2.97	0.83	0.39	6.24
Distance from market	36.98	14.33	5.00	90.00

Source: Survey results (2021)

Family size: The numbers of family size determine the amount of energy available to household. On average, sample household owned 3.94 adult equivalents ranging from 1.75 and 6 adult equivalents respectively. This implies that, there is a variation in the number of family size between sample household in the study area (Table 3).

Age of household head: Age is a proxy variable for experience of households in agricultural production. The average age of sample household was 54.56 year with minimum and maximum of 28 and 87 year respectively. This implies that the average age of sample households lies within the economically productive age groups (Table 3).

Farm size: The total farm sizes owned by households determine the amount agricultural production and hence, food security level of households. The average farm size owned by sample households 0.91 ranging from 0.5 to 3 hectare respectively (Table 3).

Livestock size: The numbers of livestock owned by households determine the amount cash available to households during food shortage and hence, enhance household access to food. The average livestock size owned by sample household was 2.97 ranging from 0.39 to 6.24 tropical livestock unit respectively (Table 3).

Distance from market: The availability of market near to household home determines household access to food grain which in turn enhances household food security. The average

distance of household home from nearest market was 36.98 walking minutes ranging from 5 and 90 walking minutes respectively (Table 3).

3.2. Institutional, social and demographic characteristics of the respondents

Access to credit: Credit enhances household access to agricultural inputs which in turn improve the agricultural production and hence, food security level of the households. The survey result depicted that, about 59.44% of the household was used credit from microfinance institution. This implies that, majority of the sample households were obtained the credit services (Table 4).

Table 4. Institutional, demographic and social characteristics of sample respondents

Variable name	Freq.	%
Access to credit (1=Yes)	233	59.44
Access to extension (1=Yes)	210	53.57
Access to saving (1=Yes)	164	41.84
Participation in community organization (1=Yes)	201	51.28
Access to off/non-farm income (1=Yes)	106	27.04
Soil fertility (1=Yes)	252	64.29
Sex of household head (1=Male)	244	62.24

Source: Survey results (2021)

Access to extension: Extension is a key to improve agricultural production and productivity which in turn improve the food availability and food security level of households. About 53.57% of the sample households were obtained the extension service while the remaining 46.43% were not obtained. This implies that, majority of the sample households were obtained the extension services (Table 4).

Access to saving: Saving improve households access to cash deposited which in turn helps to enhance the purchasing power of the households especially during the food shortage. The survey results show that, about 41.84% of households had access to money saved in microfinance institution. This implies that, majority of the sample households did not have the culture of saving money in bank/microfinance institution (Table 4).

Participation in community organization: Participation in community based organization like ikub and idir enhance households access to cash which in turn improve household purchasing power and hence, food security status. According to the results, about 51.28% of the sample households were participated in community based organization (Table 4).

Access to off/non-farm income: Access to off/non-farm income may enhance the purchasing power of the households and hence, improve production and food security status of household. The survey result depicted that, about

27.04% of the sample household had access to off/non-farm income while the remaining 72.96% had not implying that, majority of the sample household did not have access to off/non-farm income (Table 4).

Soil fertility: Household access to fertile land may enhance the amount of production and productivity of the crops and hence, improve food security of the households. The results show that, about 64.29% of the sample had access to fertile land while the remaining was not implying that majority of the land owned by households are fertile (Table 4).

Sex of household head: Being male headed is positively correlated with being food secure due to the fact that male had more access to outside information than female. The survey result shows that, about 62.24% of the sample households were male implying that majority of the sample households are male (Table 4).

3.3. Food security status of sample households in the study area

The major food grains consumed by sample households were cereals like Teff, wheat and barley and vegetables like potato, tomato, garlic and onion (Table 5). Identifying the types of food grain consumed by household was used to calculate the amount of energy available to sample households in the study area. On average, the amount of food grains consumed by sample households per year was 350.98 kg implying that the average amount of energy available to sample households was per year 59,057.85 kcal with minimum and maximum of 14,991.75 kcal and 132,682 kcal respectively (Table 6). In other words, the average amount of energy available to households per adult equivalent (AE) per day was 429.29 kcal with minimum and maximum of 93.34 kcal and 1,311.91 kcal respectively (Table 6). When compared to the threshold/standard, all sample households targeted under productive safety net programs were food insecure. This could be attributed to many socioeconomic, political, institutional, and demographic factors affecting food security.

3.4. Factors affecting rural households food insecurity in the study area

Among 13 hypothesized explanatory variables, 8 variables namely family size, sex of household head, farm size, livestock size, access to credit, participation in community organization, access to off/non-farm income, and access to saving significantly affected household food insecurity (Table 7). These significant variables are interpreted as follow.

The number family member had positive relationship with households' food insecurity at $P<0.01$. This result revealed that as the number of family members increased by one unit, food insecurity increased by 83.70 units (Table 7). This is due to the fact that large family members can compete with the existing scarce resources and hence, enhance household food insecurity. Ergando And Belete (2016) also found a positive relationship between household food insecurity and family size during their study on the analysis

of Household Food Insecurity and its Covariates in Girar Jarso district, Oromia Regional State, Ethiopia and hence, stated that with existing high rate of unemployment and less employment opportunity coupled with low wage rate payment, additional family member shares the limited resources that lead the household to become food insecure.

Table 5. Types of food grain consumed by households in the study area

Access to food grain (n=392) (1= yes; 0= No)	Freq.	%
Teff (Yes)	153	39.03
Wheat (Yes)	376	95.92
Barley (Yes)	253	65.54
Sorghum (Yes)	27	6.89
Maize (Yes)	4	1.02
Bean (Yes)	2	0.51
Pea (Yes)	3	0.77
Chickpea (Yes)	2	0.51
Oat (Yes)	2	0.51
Garlic (Yes)	392	100
Onion (Yes)	392	100
Potato (Yes)	392	100
Tomato (Yes)	392	100

Source: Survey results (2021)

Table 6. Amount of energy available to households in the study area

Variables	Mean	Std. Dev.	Min	Max
Amount of food grain available to households per year (kg)	350.98	88.30	128	805
Amount of energy available to households per year (kcal)	59,057.85	15,457.98	14,991.75	132,682
Amount of energy available to household per AE per day (kcal/AE/day)	429.29	153.03	93.34	1,311.91

Source: Survey results (2021)

A negative and significant relationship is observed between sex (male-headed) and food insecurity at P<0.1. This means as the number of male-headed households increased by one unit, the households' food insecurity decreased by 26.47 units (Table 7). This is because males headed households have high chance to participate in various income-generating activities and social organizations than female-headed. Desalegn and Yu (2017) study also found that sex of household was statistically significant and positively correlated food security ad stated that the probability of being food security is high when the household head is male due to the fact that Males have the capability to participate in various income generating activities while the female is disadvantageous because they are often limited to certain income earning activities and overloaded with households reproductive roles.

As expected, farm size had negatively and significantly affected households' food insecurity at P<0.01. This result shows that when farm size owed by sample farmers increased by one unit, food insecurity decreased by 89.83 units (Table 7). The probable reason is that households with large farm sizes had more capacity to produce and diversify crops which in turn increased the consumption and exchange of food grain which in turn decreased food insecurity. Moroda et al. (2018) on their study on the determinants of food insecurity of rural households in Boset district of Ethiopia explain that having more cultivable land is strongly associated being food secure at less than 1% significance level and hence, households with more cultivable land could produce more food, or even may diversify their crop to insure for crop failure.

The relationship between livestock size and food insecurity was negative and significant at P<0.05. This result depicts that increasing the number of livestock by one unit would result in 10.77 unit decrease in household food insecurity (Table 7). This is because livestock can be used to obtain income through exchange especially during food shortages and hence, reduce food insecurity. A similar result is also accepted by Ayalneh and Shimelis (2009) and Amare et al. (2020).

Households food insecurity is negatively affected by access to credit services at P<0.01 implying that as households' access to credit services increases by one unit, food insecurity decreases by 33.85 units (Table 7). This is because credits enhance households' participation in income generating activities and also enhance the purchasing power of the agricultural inputs. Desalegn and Yu (2017) study on Analysis of Factors Affecting Household Graduation from Ethiopian Productive Safety Net Program in Babile district, Oromia Region, Ethiopia also found that Access to credit to credit have a significant and positive relationship with households food self-sufficiency due to the fact that credit gives the households an opportunity to be involved in income generating activities which in turn enhance the financial capacity and purchasing power of the beneficiaries.

Participation in community organization was negatively affect households' food insecurity at P<0.01. This reveals

that as households' participation in community organization increased by one unit, food insecurity level of households' can be decreased by 50.04 units (Table 7). This is because community based organization enhance household access to support during food shortage and hence, reduce food insecurity. Mulu and Workneh (2017) also found positive and statistically significant relationship between participation in social organization and female household food insecurity in west Shewa zone, Oromia region, Ethiopia.

Table 7. Factors affecting food insecurity level of households in the study area

Independent Variables	Coef.	SE.	T	P>|t|
Education level of household head	3.328	4.099	0.81	0.417
Family size	-83.702***	6.576	-12.73	0.000
Sex of household head	26.474*	13.507	1.96	0.051
Age of household head	-0.591	0.407	-1.45	0.147
Farm size	89.831***	13.533	6.64	0.000
Livestock size	10.768**	5.187	2.08	0.039
Access to extension	15.298	10.779	1.42	0.157
Access to credit	33.853***	9.424	3.59	0.000
Distance from market	-0.076	0.262	-0.29	0.773
Participation in community organization	50.040***	12.358	4.05	0.000
Access to off/non-farm income	55.812***	11.597	4.81	0.000
Soil fertility	-11.735	13.445	-0.87	0.383
Access to saving	57.401***	9.532	6.02	0.000
Constant	562.006	38.326	14.66	0.000
Number of observation	392			
F(13, 378)	78.23			
R^2	0.7290			
Adjusted R^2	0.7197			

Source: Survey results (2021), SE: Standard Error R^2*: Coefficient Determination, *, ** and *** represent statistical significance at 10%, 5% and 1% respectively*

Households' access to off/non-farm income positively affected food insecurity level at P<0.01 implying that as access to off/non-farm income increased by one unit, households' food insecurity decreased by 55.81 units (Table 7). This is because access to off/non-farm income would enhance the purchasing power of the households and could serve as livelihood diversification strategies which in turn reduce food insecurity. Ergando And Belete (2016) also found positive relationship between household food security and access to off/non-farm income.

Table 8. Conversion factor of family size into adult equivalent

Age group (years)	Male	Female
<10	0.6	0.60
10-13	0.9	0.80
>13	1.0	0.75

Source: Storck et al., 1991

The relationship between household saving habits and food insecurity was negative and significant at P<0.01. This result depicts that as household saving habits increased by one unit, food insecurity of households' decreased by 57.40 units (Table 7). This is because saving increases the ability of households' to cope up with shock which may bring unexpected changes in food production, prices and income and hence, reduce food insecurity. This result is supported by the finding of Lilian et al. (2013) study on Household Food Security and Commercialization among Smallholder Farmers in Kenya stating that households' access to savings can be instrumental in raising their ability to produce food and access it from the market and hence ensuring food security.

4. Conclusion

This study aimed to measure the status of rural household food insecurity and identify its determinants in the study area. The survey result depicted that, all sample households targeted under the productive safety net program were food insecure. This means that the daily energy available to households was less than 2100 kcal per adult equivalent. This is due to lack of credit service, large family size, lack of saving habit, lack of participation in community organization, and lack of large livestock size. Therefore, in order to enhance the rural households "food security" in the study area, the policymakers should focus on the expansion of credit services, promotion of family planning, expansion of the off/non-farm activities, and enhancing the household saving habit.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Nigusu Abera: Participated in all research activities (data collection, analysis and manuscript preparation). **Shewadinber**

Mekonin: Participated in data collection and manuscript preparation.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

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Abbreviations

CSA: Central Statistics Agency

FAO: Food and Agricultural Organization

MoARD: Ministry of Agriculture and Rural Development

NSZANRO: North Shewa Zone Agriculture and Natural Resources Office

WFP: World Food Program

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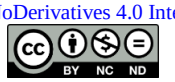

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A Review Article: With Advantages and Disadvantages The Role of Non-*Saccharomyces* Yeast in the Wine Industry

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ABSTRACT

This article aims to describe non-*Saccharomyces* yeast and their effects on wine composition, fermentation, chemistry and organoleptic characters. The use of non-*Saccharomyces* yeast is on the rise in the wine industry despite the negative perception from previous research. It is known that higher levels of non-*Saccharomyces* yeast could cause implications during winemaking practices. On the contrary, non-*Saccharomyces* yeast provides complexity, richer aroma and flavour and decreases ethanol content. If the main goal is using indigenous yeast and having a starter culture, use of non-*Saccharomyces* yeast collected from winery environment could be an option, yet again a risky option. However, previous studies indicated the relation between acetic acid production and the use of non-*Saccharomyces* yeast. In brief, it is important to increase sanitation in the winery environment and personal awareness to maximize cleanliness and to reduce any unwanted yeast activity. More importantly, in the recent years, the use of non-*Saccharomyces* yeast is attracting winemakers to achieve unique wine styles, and it is an important topic that should be taken under consideration, particularly on a research basis, specifically for targeting consumer liking-perceptions of the wine. In addition to their positive effect on sensory characters on wines, non-*Saccharomyces* yeasts as bio-control agents (BCAs) is also charming researchers around the globe.

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1. Introduction

In the late 1800's Louis Pasteur have studied yeasts and their roles in the winemaking (Jolly et al., 2014). Winemaking is a process that requires many steps and decision making to reach the best quality as final product. One of the most important of them is fermentation step and the choice of yeast. The role of the yeast is to transform sugar into alcohol, and it is depending on multiple factors such as N availability, temperature, aimed style, grape quality etc. The expectation from yeast is short lagging phase, minimum residual sugar, complexity of aroma and flavour. In addition, activity at low temperatures and less foaming are also important aspects. Besides, low temperatures could favour the growth phase of non-Saccharomyces yeast at the beginning of fermentation. In general, active dried yeast inoculation is the common method industry wise (Aranda et al., 2011). Fermentation usually begins with non-*Saccharomyces* yeast group, present on grape must or on winery environment and activity stops due to high ethanol levels approximately in 2-4 days. Moreover, *Saccharomyces cerevisae* continues and ends the fermentation after this initial process. Whereas activity of non-*Saccharomyces* yeast for couple of days at the beginning they produce important elements that effects the quality of wine (Manzanares et al., 2011).

Following research displayed that the role of *Saccharomyces cerevisae* is not only taking part in the fermentation process also affecting the aroma precursors of wine by manipulating the secondary metabolites. *Saccharomyces cerevisae* were the main specie and both indigenous and exogenous forms were desired. Therefore, grape juice contains many other yeast species known as non-Saccharomyces yeasts. Latest technology helped the researchers to isolate good characteristics of non-*Saccharomyces* yeasts. Approximately 40 non-*Saccharomyces* species discovered and have been cultured in-vitro conditions derived from grapes and grape juices. Grape berries are the main foundation of these yeasts whilst non-*Saccharomyces* yeast existence in the vineyard is not a common situation (Hranilovic, 2018). Some of the important non-Saccharomyces yeasts found in vineyards and wineries can be listed as; *Aureobasidium, Brettanomyces, Debaryomyces, Hanseniaspora, Metschnikowia, Lachancea, Torulaspora. Pichia, Rhodotorula, Starmerella and Zygosaccharomyces.* Also, non-*Saccharomyces* yeasts *Torulaspora delbrueckii, Metschnikowia pulcherrima* and *Pichia kluyveri* is preferred for sparkling wine production recently (Ivit and Kemp, 2018). Compared to *Saccharomyces cerevisae*, these species usually present in grape juice naturally and abundant in population which

makes them viable against *Saccharomyces cerevisae* dominance (Jolly et al., 2014). As a winemaking term, non-*Saccharomyces* yeast designates multiple yeast types other than *Saccharomyces cerevisae* which they are present in wineries and around.

2. Advantages and Disadvantages

There are negative aspects of using non-*Saccharomyces* yeasts as well as positive aspects (Ivit and Kemp, 2018). Previous research was claiming non-*Saccharomyces* yeast negatively affected by added SO_2 and yeast growth controlled by the addition (Jolly et al., 2014). Presence of oxygen is critical for the lifespan and durability of some species such as *Lachancea thermotolerans* and *Torulospora delbreuckii* as well as cell viability of non-*Saccharomyces* species (Hranilovic, 2018). Therefore, late publications revealed that their intolerance were due to dual toxic effect between SO_2 and fermentation derived alcohol. However, spontaneous ferments have led unique, fine textured and more complex wines however with the threat of microbial spoilage (Jolly et al., 2014).

2.1. Alcohol

The modern research technique called cell counting arose the re-questioning of non-*Saccharomyces* yeasts' role. In winemaking higher alcohol levels are not desirable and non-*Saccharomyces* yeasts generally decreases the level of high alcohols however there is also a major problem of variability between species (Jolly et al., 2014). In addition, each non-*Saccharomyces* yeast produce diverse levels of alcohol during fermentation. A non-*Saccharomyces* yeast *Metshnikowia pulcherrima* has a key prospect for producing lower alcohol wines (Hranilovic et al., 2020) and some strains of *M. pulcherrima* are now available for wine industry (Aplin et al., 2021).

Decline of alcohol content could range between 0.6-1.2% (v/v) (Hranilovic et al., 2020). *M. pulcherrima* and sequential inoculation of *M. pulcherrima* and non-*Saccharomyces* did not alter sensory properties but provided lower alcohol levels on final wines (Aplin et al., 2021).

Glycerol is another important yeast metabolite occurs during alcoholic fermentation in wine. It has positive effect on smooth mouth feel, sweetness and enhances the complexity of the wine. While this is the case, there is a positive correlation between glycerol and acetic acid production, which could be unfavourable for final wine quality (Jolly et al., 2014).

2.2. Flavour, aroma, mouthfeel and colour

There is a wide choice of flavour compounds by non-*Saccharomyces* yeast according to past publications. Some metabolites affected by non-*Saccharomyces* yeast activity, such as terpenes, esters, alcohols, glycerol, acetaldehyde, acetic and succinic acid. Some specific aroma and flavour compounds are existing in grape berries as non-flavoured or non-aromatic precursors. These compounds require a specific enzyme β-glucosidae to hydrolyze and form free volatile compounds (Jolly et al., 2014). Although, there are non-*Saccharomyces* species such as *Debaryomyces*, *Hansenula*, *Candida*, *Pichia* and *Kloeckera* that displays different levels of β-glucosidae activity and improve aroma and flavour of wine by freeing volatile compounds. In another trial, addition of an enzyme taken out from *Debaryomyces pseudopolymorphus* has enhanced the volatile terpenes in Riesling, Muscat and *Arien varities* (Jolly et al., 2014). Interactions between *Saccharomyces* and non-*Saccharomyces* yeasts, regardless of positive and/or negative, significantly effects the foundation of aroma compounds. Consequently, co-inoculation with multiple non- *Saccharomyces* species is a likely way to enhance the aroma multiformity and the quality of the final product. Therefore, this can enthuse winemakers to create optimum multiple ferment starters to enhance the volatile compounds and organoleptic attributes of local wine industry (Zhang et al., 2022).

Table 1. Sensory characters and their technical effects of some non-*Saccharomyces* species

Non-*Saccharomyces* Species	Sensory Character	Technical Effect
Hanseniaspora/Kloeckera	Floral, rose petal notes	Increases floral notes 2-10x compared to *S. cerevisae*
Hanseniaspora vineae	Floral jasmine	Floral
Lachancea thermotolerans	Floral, rose petal, strawberry, citrus hints	Slight alcohol reduction
Metschnikowia pulcherrima	Rose, Floral aromas	Enhanced varietal aromas
Pichia kluyveri	Grapefruit, passionfruit	Fruit aromas
S. pombe	Stable pigments and colour, vitisin A precursor, silky astringency	Improve of visitin A activity
Torulospora delbrueckii	Floral, honey, apple, black currant	Dark fruit aromas
Wickerhamomyces anomalus	Floral, honey, banana, fruity	Increases fruity aromas

(Adapted from Morata et al., 2019)

The bacteria *Oenococcus oeni* is the key factor for a successful malolactic fermentation (MLF) in wine (Lonvaud-Funel, 1999; Balmaseda et al., 2021). However, physicochemical attributes of wine make it a rough

environment for *Oenococcus oeni* to dodge a couple of stress factors (Bech-Terkilsen et al., 2020). Therefore, specific loci identified in *Oenococcus oeni*, then again dependent on strain, due to the metabolic abilities of the yeasts, has a major effect on *Oenococcus oeni* to acclimatize to wine environment. For instance, study of Balmaseda et al., 2021, revealed linkage of yeast-bacteria interaction and their harmony during Malo-lactic fermentation (MLF) in which resulted as the use of non-*Saccharomyces* yeast triggered a faster paced MLF.

Even if there are not enough studies for colour effect of non-*Saccharomyces* yeast, it is known that yeast has an impact on wine colour (Jolly et al., 2014). A non-*Saccharomyces* species *Pichia guilliermondii* has provided better colour stability and well-formed anthocyanin molecules as well as *S. cerevisae* (Benito et al., 2011). Mostly, flavour compounds primarily derived from grape berries and secondarily from esters by yeast activity throughout fermentation. There are some strains of *S. cerevisae* which are varying by their tannin binding attraction (Mazauric and Salmon 2006; Sidari et al., 2007; Hranilovic et al., 2018). The ability of tannin binding of a yeast can be sturdily affected by the structure of the fermentation environment (Rinaldi et al., 2016; Hranilovic et al., 2018). Božič et al. (2021) have investigated the effect of indigenous yeast on colour of Pinot Noir wines. According to their study, the use of indigenous yeast had an effect on chemical attributes of wines, along with sensory characters. Even though more intense colour is achievable via indigenous strains, factors such yeast interaction, unwanted metabolite secretion needs to be taken into consideration when choosing fitting starter cultures since they might decrease the wine quality.

2.3. Risks and benefits

New inoculation methods which involve non-*Saccharomyces* yeasts often leads to slow paced fermentations, changes in wine structure and quality enhancement (Hranilovic et al., 2018). According to previous publications, as an alternative to *S. cerevisae* yeast, there are several roles of non-*Saccharomyces* yeast in winemaking which are increase of complexity, flavour, aroma, decrease of alcohol levels, reaching a better colour. The sequential inoculation of *Starmerella bacilliaris* with S. cerevisiae declines the acetic acid levels in sweet style wines owing to the growth performance of the *S. bacilliaris* even at peak sugar levels (Rantsiou et al., 2012). Moreover, invertase activity of *S. bacilliaris* strains revealed that, it can consume sugars other than glucose during the fermentation. However, hydrolytic enzyme activities of non-*Saccharomyces* yeast such as glucosidases, lipases and proteases which are the causes of arose of volatile and non-volatile by-products (Genc, 2022). Moreover, Barbosa et al. (2022) have investigated the supervised and unsupervised machine learning to modulate fermentation environment. According to their study, it was achievable to improve aromatic and fermentation prospect of *H. guillermondii* UTAD222 strain by temperature, nitrogen (N) and/or sugar modifications which then directly involved in production of specific volatiles. Due to use of non-*Saccharomyces* yeast such as Brett or high amounts of volatile aroma compounds may cause reductions in wine. In addition, malic acid in wine is linked to tough mouthfeel characteristics and decrease towards ripening stage (Ribéreau-Gayon et al., 2006; Su et al., 2014; Hranilovic et al., 2018). However, *Schizosaccharomyces pombe* can completely break down malic acid throughout the fermentation whereas other species takes part of its fractional break down or an incline in concentration, reliant on the strain and environment (Kapsopoulou et al., 2007; Jolly et al., 2014; Su et al., 2014; Benito et al., 2015; Hranilovic et al., 2018). Sidari et al. (2021) have tested the fermentation performances of eleven yeast strains. Non-*Saccharomyces* yeasts have indicated lower fermentation vigour compared to *S. cerevisae* yeasts which were performed the peak performance. In the same study, alike observations were defined for *M. pulcherrima* 125/4 strain and worst performance defined for *D. hansenii* 5-1-6 strain.

Milestone studies about *S. cerevisae* up to date is essential to illustrate the identified and/or potentially unique pathways of aromatic compound metabolism in non-*Saccharomyces* yeasts (Hazelwood et al., 2008; Sumby et al., 2010; Hranilovic et al., 2018). Research from Italy have shown 58 samples isolated from various winery environments including winery equipment, grapes and musts. These winery surfaces hold the microorganisms which starts fermentations (Ciani et al., 2004). In a perfect world, *S. cerevisae* is enough to start and end fermentation but research shows that it causes uniformity. In addition, lack of non-*Saccharomyces* yeasts may cause less complexity, flavour and aroma characteristics (Ivit and Kemp, 2018).

A traditional method known as pied de-cuve is used to acquaint a chosen yeast or start a fermentation from an already fermenting grape must. Non inoculated pied de-cuve, which can be obtained started from early-stage fermenting grape musts, could be a potential method if the aim is to trigger the native microflora. This method could enhance the microbiological control during ongoing alcoholic fermentation, in comparison to spontaneous fermentation whilst conserving the typicity and terroir of the final product (Mas and Portillo, 2022)

Ocón et al. (2010) have discovered yeasts on winery environment nearly 40% of it were *S. cerevisae* and suggested that it is more tolerant to use of SO_2 compared to non-*Saccharomyces* yeasts. In addition, yeast colony presence is also depending on the season, grape cleanliness as well as winery cleanliness. Non-*Saccharomyces* yeasts are much stronger compared to *S. cerevisae* under general methods of sanitation and as a result population of non-*Saccharomyces* yeasts were higher than *S. cerevisae* (Ocón et al., 2010).

The organic acids, as an outcome of some non-*Saccharomyces* yeast activity, aids to obtain sounder and more stable wines even during the process of ageing both in barrel and bottle. Moreover, the biocontrol attributes of these yeasts provide a harmless production line for wines by the decreasing the levels of wine preservatives, e.g. sulphites in different forms (Morata et al., 2019). Equally important, non-*Saccharomyces* strains consist of a major variety of species that have indicated effectual antagonism against pathogenic fungi on grapevines. Several studies have revealed the capabilities by further increasing its act by linking it with other components or organisms. Furthermore,

endophytic yeasts could shortly become a supply source as bio-control agents (BCAs). In fact, *Metschnikowia*, *Pichia*, and *Hanseniaspora* have recently been discovered in grapes (Di Canito et al., 2021). In a similar study, according to Agarbati et al. (2022), *A. pullulans* and *M. pulcherrima* have shown the most favourable performances against *Botrytis cinerea* (grey mould). This method is closely linked to consumer expectancies while taken advance of bio-control applications into account in agriculture and food industry, based on ecological welcoming and chemical free treatments.

3. Conclusion

Regardless of growing numbers of research focusing on identification of various non-*Saccharomyces* yeast species and strains, their prospective effects on wine quality widely undiscovered (Hranilovic, 2018). Non-*Saccharomyces* yeast known to be vulnerable against some stress factors and not able to metabolize all sugars during fermentation contrasting *S. cerevisae* (Jolly et al., 2014; Hranilovic, 2018) However, to overcome inconsistency and declined complexity while avoiding the risks of naturally inoculated fermentation, mixed fermentations could be an option in vinification (Ciani et al., 2010; Hranilovic, 2018). Therefore, use of mixed starter culture with non-*Saccharomyces* is becoming a new trend recently (Aranda et al., 2011).

To summarize, factors such as sanitation practices in the winery environment, tools used in the vineyard, transport sanitation including equipment operations and personal awareness has a vital role to avoid unwanted yeasts. Sanitation frequency and quality of sanitation products are equally important facts. Equally important, *S. cerevisae* and non-*Saccharomyces* species found in the wineries could be a source for indigenous yeast cultures for both scientific purposes and small batch winemaking for further research to prove their effectiveness and investigate quality alterations in the world but most specifically in Türkiye.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Metehan Gunhan: Validation, Writing - Original draft, Visualization, Investigation, Review

Ethical approval

Not applicable.

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Determination of agricultural and technological characteristics of different lavender (*Lavandula angustofolia* Mill.) genotypes in ecological conditions of Çorum province *

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* This study is derived from Mustafa AKDOGAN's doctoral thesis.

ABSTRACT

This study aims to determine the agricultural and technological properties of *Lavandula angustofolia* Mill. varieties under the ecological conditions of Çorum province from 2019 to 2020. Raya, Munstead, Silver, Sevtapolis and Vera lavender varieties collected from the Sungurlu district of Çorum province were used in the study. The highest plant length (60.27-68.43 cm) was obtained from Silver cv. in both years. The best fresh stem flower yield was obtained from Mustead cv. (479.2 kgda^{-1}) in 2019 and Sevtopolis cv. (545.6 kgda^{-1}) in 2020. Raya and Mustead cv. Because of these varieties are registered; after the variety name, the cv. shortening is not required gave the highest essential oil ratio in both years. In addition, it was observed that the Sevtapolis variety had the highest linalool ratio (49.07%). Also, Mustead and Sevtapolis varieties stood out in their fresh flower yield and essential oil quality.

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1. Introduction

Lamiaceae or *Labiatae* family is an extensive family containing perennial, herbaceous plants, shrubs and trees, with about 236 genera and around 7173 taxa. Species belonging to the *Lamiaceae* family spread over many continents globally and can grow in different ecological regions and distinct areas, at different altitudes and in different habitats. The *Lavandula* genus is an essential member of the *Lamiaceae* family and includes 39 known species (Paton et al., 2004). The *Lavandula* genus geographically spreads from Europe, Southwest Asia, Arabian Peninsula, North and South America and North Atlantic Islands to India via the Mediterranean Basin (Upson and Andrews 2004; Aprotosoaie et al., 2017). The genus *Lavandula* includes annual or short-lived herbaceous perennials, shrub-like perennials, short bushes or shrubs (Upson and Andrews 2004). Many members of the genus are grown extensively for their essential oils commercially and ornamentals for garden and landscape use in temperate climates. *Lavandula* species are generally known for their multiple pharmacological effects sedative, antispasmodic, anticonvulsant, analgesic, antioxidant and local anesthetic activity. They are also used for medicinal purposes (Kovatcheva et al., 2001; Kageyama et al., 2012).

The essential oils found in most of the *Lavandula* species contain 50-60 compounds consisting of monoterpenes and sesquiterpenes. These terpenes are compounds commonly found in these plants that perform important ecological functions such as repelling insects and suppressing the growth and development of other competitive plants (Gershenzone and Croteau 1991; Southwell et al., 2003; Franks et al., 2012). *Lavandula angustofolia* (lavender) *Lavandula* x intermedia (lavandin), *Lavandula latifolia* (*Lavandula spica*; spike lavender) and *Lavandula stoechas* (Spanish lavender) are widely used in medicine, food, cosmetics, perfumery and aromatherapy (Vairinhos and Miguel, 2020). Both lavender and lavandin essential oils have wide applications in a various industrial products including perfumes, pharmaceuticals, cosmetics, and personal care and home care products (Cavanagh and Wilkinson, 2002; Lesage-Meessen et al., 2015). Lavender oil content and species characteristics depend on many factors such as genotype, differences between varieties and their hybrids, climatic data, agronomic factors, and processing and storage of raw plant materials (Morgan et al., 2006; Mantovani et al., 2013; Golubkina et al., 2020). Many agronomic studies have been carried out with lavender species, and there are production and agronomic studies

under organic agriculture or good agricultural practices. Many scientific studies have been conducted on yield, yield components and essential oil content in fertilization and plant density under different organic production conditions (Renaud et al., 2001). In this regard, it is of great value to analyze lavender species with economic importance in different environmental conditions and to determine especially *Lavandula angustofolia* varieties with high yield and quality that can adapt to the ecological conditions of Çorum province. Therefore, this study aims to identify the agricultural and technological characteristics of some lavender varieties of the high commercial value used for the first time within the regional conditions and determine the genotypes with high flower and essential oil yields of high adaptability.

2. Materials and methods

2.1 Plant materials and supply

The plant material of the study comprised the open rooted cuttings of Raya, Munstead, Silver, Sevtapolis and Vera varieties belonging to *Lavandula angustofolia* Mill.

2.2. Location and terrain characteristics of the trial

This study was carried out for three years (2018 was the year of establishment) between 2018 and 2020, in Çorum Province Sungurlu District Çavuş Village Toytepesi Location 0 Island 1031 parcel. The experimental area was located in the semi-arid climate zone and in terms of climate and soil conditions, it was established by planting open root seedlings in October 2018. 2018, the year of the first establishment, was not evaluated and the research was evaluated in the light of the findings that included the second year (2019) and third year (2020).

2.3. Soil characteristics of the trial field

Samples were taken from different parts of the field where the study was carried out. The slope of the trial area was very low, and there was no drainage problem. The experimental area had a clay-loam structure, and the soil pH 8.05. The findings showed that the area was slightly alkaline, the level of lime was moderate, the potassium level was good, the amount of phosphorus was sufficient and the amount of organic matter in potassium was low.

2.4. Climatic characteristics of the trial area

Table 1 shows the averages of the climate data belonging to a long-term period in the region, the monthly temperature (°C), relative humidity (%) and precipitation (mm) values during the vegetation period of the trial years.

The monthly total precipitation, average temperature and relative humidity data 2019-2020 are presented in Table 1. The total precipitation of 438 mm in the first year (2019) vegetation period was similar to the long-term average, In the 2nd year (2020), it was well below the average of 267.6 mm for long years and 2019. Especially in August 2020, precipitation of 0.0 mm was seen as an extreme situation. The average monthly temperature in the first year (2019) and the second year (2020) is similar to the long-term average, and it has been observed that the average relative humidity is higher than the long-term average in the first year and lower than the long-term average in the second year.

Table 1. Some climates data in 2019, 2020 and long-term period

Climate data	Year	Months												Total / Average
		Jan.	Feb.	Mar.	Apr.	May.	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Average temperature (°C)	2019	0.5	4.6	6.4	10.4	17.7	21.9	21.9	22.7	19.2	16.1	8.5	3.3	153.2 / 12.76
	2020	1.2	3.2	8.7	10.2	16.3	20.4	24.9	23.7	22.7	18.0	5.6	4.3	159.2 / 13.26
	Long -term (2013-19)	0.9	5.0	8.1	12.2	17.0	20.7	23.8	24.4	20.2	13.9	7.4	1.7	155.3 / 12.9
Total Precipitation (mm)	2019	51.3	30.6	17.6	56.8	24.0	125.2	30.2	35.7	2.1	0.3	35.0	29.7	438.5 / 36.6
	2020	21.6	67.5	13.9	21.3	37.9	90.4	7.9	0.0	5.5	5.2	14.5	9.7	295.4 / 24.6
	Long -term (2013-19)	42.07	21.54	53.43	30.2	63.89	88.31	1.8	14.81	14.53	24.16	28.91	32.81	416.5 / 34.7
Average Relative Humudity (%)	2019	88.6	78.7	63.4	69.4	61.2	69.3	58.1	61.0	56.7	60.4	70.1	91.7	828.6 / 69.03
	2020	84.5	79.5	69.3	64.6	63.7	66.3	54.0	37.0	42.5	40.1	61.7	68.4	731.6 / 61
	Long -term (2013-19)	83.5	71.3	64.2	57.66	61.6	60.9	48.6	49.3	49.1	60.4	67.6	83.7	757.8 / 63.1

Source: Meteorological Service

2.5. Planting

The research field trial was established in October 2018, and the unsprouted seedlings were replanted in April 2019. Open-rooted varieties of 15-20 cm tall were planted in the holes with 10 cm depth each, and the length between rows was 0.5 m and the length between furrows was 1.4 m. Totally 1330 plants were planted in 1 decare. A total of 450 seedlings were planted, 90 from each of the 5 varieties.

2.6. Fertilization

The research field trial was established in October 2018, and Fertilization is one of the critical parameters affecting the yield and quality of lavender. After planting in the trial field, seedlings were fertilized with 6 $kgda^{-1}$ of pure nitrogen (N) and 6 $kgda^{-1}$ of P_2O_5.

2.7. Essential oil obtaining, and essential oil components analysis

Fresh stemmed and dried lavender flowers were brought to the laboratory after harvest, 100 g of lavender flowers were added together with 1 liter of distilled water and placed in a Clevenger flask. The essential oil of the plant materials was obtained by hydro-distillation using Clevenger apparatus. The distillation process was terminated when it was observed that the amount of oil collected in the metered section did not change, and then the amount of essential oil was determined.

2.8. Essential oil components analysis

GC analysis of the essential oils from lavender flowers was performed using Agilent 6890N Network GC and 5973 Network mass selective detector GC-MS system. The analysis was performed using HP -Innowax column (60.0 m × 0.25 mm × 0.25 mm) (Agilent Technologies, USA) and helium as the carrier gas (1.2 mL min^{-1}). The operating conditions were: The oven temperature was set at 60°C for 10 min after injection, then increased to 220°C with a heating ramp of 4°C for 10 min, and then increased to 240°C with a heating ramp of 1°C min^{-1} without holding; the injector and detector (FID) temperatures were 250°C; the split ratio was set at 50:1; the injection volume was 2.0 µL. MS conditions were as follows: Ionization energy 70 eV; ion source temperature 280°C; interface temperature 250°C; mass range 34-450 atomic mass units. The compounds were identified by comparing their relative retention indices and mass spectra to those found in the literature, as well as their mass spectra to those found in the Wiley and Nist libraries. The percentages of the components were calculated from the GC peak areas using the normalization method

2.9. The statistical evaluation of trial design and trial results

The experiment was planned and established in a randomized block design with three replications. The variance analysis (VA) of the data obtained as a result of measurements and analyzes from the research was performed using the MSTAT-C (Michigan State University, version 2.10) computer package program. Differences and importance levels between lavender species and varieties were determined with MSD (Minimum Significant Difference) Test.

3. Results and discussion

Variance analysis was carried out using the plant height, the number of flower heads number of flowers per spike, fresh stem flower yield ($kgda^{-1}$), and fresh stem flower essential oil ratio (%) of lavender varieties in 2019 and 2020. The differences in the number of flower heads (pieces), number of flowers per spike (pieces), fresh stem flower yield ($kgda^{-1}$), and fresh stem flower essential oil ratio (%) of the varieties were found to be significant in both 2019 and 2020 at the level of $p \leq 0.01$. The following table (Table 2) presents the significance levels of the differences between the averages of these characteristics and the results of the MSD test. The average plant height of lavender genotypes showed a significant change in 2019, and it was found to be between 44.83-60.27 cm. The longest plant height (60.27 cm) was determined in the Silver variety. Also, the plant height of other varieties is between 43.93-46.93 cm. In 2020, the highest plant height (68.43 cm) was determined in the Silver variety and the lowest plant height (46.10 cm) was determined in Raya variety. Silver variety gave the highest value in terms of plant height in both 2019 and 2020. In different studies, it has been reported that the plant height of lavender varies between 60.4-69.5 cm (Arabaci and Bayram, 2005) and 46.1-59.8 cm (Atalay, 2008). In a similar study, the height of lavender varieties varied between 62.2-81.1 cm and the highest plant height (86.2 cm) among lavender varieties was determined in the Vera variety. Moreover, the lowest plant height (63.2 cm) was found in the Munstead variety. It was observed that the plant height of the early Raya and Munstead varieties was significantly shorter than the late maturing genotypes tested in each year of the two-year study and for an average of two years. In another study, no significant difference was found between lavender varieties in plant height, but plant height varied between 29.30-31.15 cm. It was observed that the Grosso Tina variety had the highest plant height (Balyemez, 2014). In a spiking study, plant height was found between 33.63-42.66 cm in L. angustifolia variety (Balcı, 2019). In this study, it was seen that the varieties were among the values reported in the literature in terms of plant height. In the study conducted by Özyazıcı and Kevseroğlu (2019), the plant height *in Lavandula angustofolia* varied between 30.00-40.60 cm, and the highest plant height was determined in the complete flowering phases.

The number of flower heads belonging to lavender genotypes showed a significant change in 2019, the number of flower heads per plant varied between 152.2-528.2. The highest number of flower heads per plant (528.2 pieces) was reached in the Sevtopolis variety, and the lowest number of flower heads per plant (152.2 pieces) was reached in the Silver variety. In 2020, the number of flower heads varied between 164.1 and 545.3 pieces. The highest number of flower heads (545.3 pieces) was determined this time in the Munstead variety, and the lowest number of flower heads (164.1 pieces) was determined in the Silver variety. Munstead varieties in both years and Sevtapolis varieties in 2019 stood out regarding number of flower heads. In a study, the highest flower spike (1217.8 pieces $plant^{-1}$) was found in the Raya variety compared to an average of two years, and the lowest flower spike (632.1 pieces $plant^{-1}$) in the Silver variety. The number of flower spikes of Sevtapolis and Munstead varieties was significantly higher, while the number of flower heads of the Silver variety was significantly low. When the varieties were compared in the study, Silver and Munstead varieties showed parallelism with our findings regarding their performance. However, it is seen that the varieties give lower values in terms of flower head numbers. The average number of flowers per spike of lavender genotypes showed significant changes in 2019 and 2020. In 2019, the number of flowers per spike varied between 36.10-54.40 per plant.

The highest number of flowers per spike (54.40 pieces) was determined in the Silver variety, and the lowest number of flowers per spike (36.10 pieces) was determined in the Munstead variety. In 2020, the number of flowers per spike varied between 38.00-68.07. The highest number of flowers per spike (68.07 pieces) was again in the Silver variety, and the lowest number of flowers per spike (38.0 pieces) was determined in Munstead variety. The Silver variety stood out in both years regarding the number of flowers per spike, followed by Vera. In another similar study, it was determined that the Silver variety had the highest number of flowers per spike (64.1 pieces) among lavender varieties at an average of two years. The least number of flowers per spike (29.9 pieces) was determined in the Munstead variety. The number of flowers per spike of the short and early Raya and Munstead lavender varieties was lower than the late varieties. Average fresh stem flower yield values ($kgda^{-1}$) of lavender genotypes also showed significant changes in 2019

and 2020. In 2019, the flower yield with fresh stems was 377.7-479.2 (kgda^{-1}). The highest fresh stem flower yield (479.2 kgda^{-1}) and flower yield per plant (g plant^{-1}) were determined in the Mustead variety, and the lowest fresh stem flower yield (377.7 kgda^{-1}) was found in the Silver variety.

Table 2. MSD Test results of some essential agricultural characteristics of lavender varieties

Lavender varieties	Plant height (cm)		Number of flower ears (piece)		Number of flowers per spike (piece)		Fresh stem flower yield (kgda^{-1})		Fresh stem flower essential oil ratio (%)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Raya	45.57 b	46.10 c	191.6 c	199.4 d	43.93 c	44.60 d	425.9 d	477.5 c	1.370 a	1.393 a
Vera	43.93 b	49.17 b	356.9 b	366.9 c	50.57 b	54.33 b	440.1 c	450.8 d	1.190 bc	1.190 c
Munstead	46.93 b	49.47 b	523.3 a	545.3 a	36.10 d	38.00 e	479.2 a	498.8 b	1.367 a	1.403 a
Silver	60.27 a	68.43 a	152.2 d	164.1 e	54.40 a	68.07 a	377.7 e	388.8 e	1.247 b	1.307 b
Sevtopolis	44.83 b	46.20 c	528.2 a	536.5 b	46.73 c	49.40 c	454.4 b	545.6 a	1.157 c	1.197 c
MSD (0.05)	5.665	0.822	6.762	6.782	3.195	3.346	5.669	10.81	0.084	0.059

The letters in the same column represent the statistically ($p \leq 0.05$) different groups

In 2020, the fresh stem flower yield was determined as 388.8-545.6 kgda^{-1}, while the best fresh stem flower yield (545.6 kgda^{-1}) was again in the flower yield per plant (g plant^{-1}), the lowest fresh stem flower yield (388.8 kgda^{-1}) was similarly determined in Silver variety. Silver variety gave the lowest fresh stem flower yield values in 2019 and 2020. In both years, it was observed that Mustead and Sevtopolis varieties stood out in terms of fresh stem flower yield (kgda^{-1}), and the results in flower yield per plant (g plant^{-1}). In a similar study, the lavander's highest fresh flower yield was 556.7 kgda^{-1} in the first year and 1499.0 kgda^{-1} in the third year (Arabaci and Bayram 2005). According to the combined results of two years in another fertilization experiment, the highest fresh flower yield (378.22 kgda^{-1}) in lavender was obtained when 2.5 kgda^{-1} nitrogen was applied (Atalay, 2008). In another study conducted in Isparta province, Raya, Munstead, Silver and Vera lavender varieties were used as materials. A high fresh stem flower yield was obtained from the 597.2 kgda^{-1} Raya variety compared to the two-year average, followed by Vera (569.5 kgda^{-1}), Mustead (499.2 kgda^{-1}), and Silver (476.2 kgda^{-1}) varieties. Karık et al. (2017) found the highest fresh flower yield in Munstead (232.87 kgda^{-1}) and Hidcote (186.87 kgda^{-1}) genotypes in their study. In another study conducted in Adana ecological conditions, the fresh flower yield of *Lavandula angustofolia* Mill. was determined as 14.27-18.66 kgda^{-1} according to the data obtained in the first year, and this low yield was attributed to the first planting year (Balcı 2019). Compared with all these previous studies, it is clear that Sevtopolis and Mustead varieties used in this study gave satisfactory results in flower yields. The fresh stem flower essential oil ratio (%) of lavender genotypes in the mean years showed a significant change in 2019 and 2020. The essential oil rate of fresh stem flowers in 2019 was determined as 1.157-1.370%. Raya variety gave the highest fresh stem flower essential oil ratio (1.370), followed by the Mustead variety with 1.367%. On the other hand, the Sevtopolis variety with 1.157 % was the lowest amount.

In 2020, the fresh stem flower essential oil rate changed between 1.190-1.403%, and the highest fresh stem flower essential oil rate was found in the Mustead variety with 1.403%. As of 2019, the lowest essential oil rate (1.197%) was determined in the Sevtopolis variety in 2020. Mustead and Raya varieties stood out in terms of fresh stem flower essential oil content, in both years, Sevtopolis variety contained the least essential oil content in 2019 and 2020. The total number of 39 essential oil components in fresh flowers was determined in lavander genotypes (Table 3). In fresh lavender flowers, 33 essential oil components were determined in Raya, Silver and Vera varieties, 34 number in Mustead and 28 in Sevtapolis variety.

Linalol and linalyl acetate were the most fundamental compounds in lavender genotypes. These compounds were followed by lavandulyl acetate, cis-β-ocimene and geranyl acetate. The highest linalool rate (49.071%) was found in the Sevtopolis variety compared with the other lavender varieties whereas the lowest linalool rate (24.186%) was determined in the Silver variety. In terms of linalyl acetate, the highest linalyl acetate ratio (29.993%) was detected in the Raya variety, while the lowest rate (20.959%) was detected in the Silver variety once again.

The highest lavandulyl acetate ratio (8.817%) was determined in the Raya variety, followed by Munstead with 8.148% and Sevtopolis variety with 5.662%. It was found that Raya and Silver equivalents had deficient (less than 2%) lavandulyl acetate content. Except for the Silver variety, the findings showed that the other varieties contained significant amount of geranyl acetate. The content of these four varieties varied between 2.874 and 3.528%, and the highest geranyl acetate ratio was again found in the Sevtopolis variety. According to European (EU) and American and British (USP and BP) ISO 3515:2002 Lavender Oil Quality Standards, linalool and linalyl acetate cannot be less than 20-25% to be evaluated in the cosmetics industry. Accordingly, it is seen that all varieties approach and exceed this limit. It is perceived that the varieties are mainly in compliance with the standards. It was determined that the Silver variety, remained within the lower limits according to linalool and linalyl acetate ratio standards. According to European (EU) and American and British (USP and BP) ISO 3515:2002 standards, the ratio of 1,8-cineole should be below 2.5%, while it was determined that all varieties were in compliance, except Silver variety. It was determined that four varieties had a content varying between 0.282-0.367% in terms of camphor ratio, and the Silver variety contained 6.899% camphor. Similarly, it should be below 0.5% according to European (EU) and American and British (USP and BP) ISO 3515:2002 standards. Except for the Silver variety, all

varieties have camphor content in accordance with this standard, while the Silver variety contains 6.899% camphor. In a study conducted by (Karadogan et al., 2003), in the areas where lavender is grown in the lakes region, in terms of essential oil components, it was determined that lavender contains in 43.1% linalool, 22.3 linalyl acetate, 3.8% citronellol, 6.8% camphor and 0.2% borneol, respectively (Karadogan et al., 2003).

Table 3. Fresh stem flower essential oil components of Lavender varieties (%)

Substance name	Raya	Vera	Munstead	Silver	Sevtapolis
α-pinene	0.181	0.200	0.195	0.648	0.088
α- thujene	0.067	0.059	0.045	0.063	0.024
camphene	0.147	0.243	0.196	0.365	0.125
β-pinene	-	0.054	0.029	1.119	tr
sabinene	0.015	0.038	0.025	0.597	tr
Δ-3-carene	0.090	0.188	0.171	0.886	0.081
β-myrcene	1.123	1.128	1.134	1.425	1.115
α-terpinene	0.043	-	0.044	-	tr
limonene	0.408	0.508	0.498	2.905	0.435
1,8-cineole	0.733	1.318	0.701	20.988	0.407
phellandrene	-	-	-	3.544	-
cis-*β*-ocimene	11.502	11.631	13.518	6.030	2.275
γ-terpinene	0.089	0.146	0.170	0.120	-
trans-*β*-ocimene	4.807	4.684	5.168	1.569	2.361
3-octanone	0.312	0.346	0.217	-	-
hexyl acetate	0.196	0.081	0.101	0.035	-
p-cymene	0.343	0.584	0.435	0.400	0.813
α-terpinolene	0.257	0.246	0.245	0.239	0.229
hexyl propionate	0.016	0.010	0.012	0.020	0.019
hexyl isobutyrate	0.039	0.040	0.033	0.081	0.039
1-octen-3-il-acetate	1.188	1.110	1.269	0.029	1.029
rosefuran	0.016	0.030	0.027	0.036	0.012
hexyl butyrate	0.234	0.233	0.225	0.523	0.333
hexyl-2-methyl butyrate	tr	0.017	-	0.170	tr
cis-linalool oxide	0.121	0.137	0.108	0.034	0.211
trans-linalool oxide	0.109	0.109	0.103	0.108	0.177
camphor	0.282	0.367	0.251	6.899	0.290
linalool	30.868	34.473	31.894	24.186	49.071
linalyl acetate	29.993	24.370	25.738	20.959	25.389
bornyl acetate	0.256		0.545	-	0.202
lavandulyl acetate	8.817	0.413	8.148	1.496	5.662
caryophyllene	0.842	1.134	1.213	0.276	1.487
β-farnesene	1.305	1.717	2.328	0.793	1.073
neryl acetate	1.816	1.669	1.232	0.246	2.067
geranyl isovalerate	-	-	-	0.981	-
geranyl acetate	3.077	3.187	2.874	-	3.528
cuminal	0.071	0.050	0.063	0.355	0.152
caryophyllene oxide	0.141	0.204	0.108	0.139	0.441

In another study conducted in different regions in Greece, differences in essential oil composition of *L. angustifolia* genotypes were evaluated; in the Ist Region, the main compounds were determined as linalyl acetate (30.62%), linalool (29.56%), 1,8-cineole (5.18%), and camphor (4.03%). The main compounds for the IInd Region were linalyl acetate (26.92%), linalool (16.78%), 1,8-cineole (15.55%), and camphor (7.41%), (Hassiotis et al., 2010). Kara and Baydar (2013), in their study with lavender and lavandin genotypes in Isparta province, found the highest linalool content (43.3%) in fresh flowers from the Dutch variety in the first year and Vera (43.9%) in the second year. The highest linalyl acetate ratio was found in Super A variety, the highest camphor content in the first year than Super A (19.8%) in the second year and Dutch (10.0%). Dutch variety (46.5% and 47.0%, respectively) gave the highest linalool content, and the Super A variety (32.8% and 29.5%, respectively) gave the highest linalyl acetate content in dry sessile flowers in both years. In 2009 and 2010, the highest camphor content was determined at Silver (12.6%), in the first year and the Dutch (10.9), in the second year. In the study carried out to determine the morphological, yield and quality characteristics of lavender (*Lavandula* species and varieties in the Menemen district of İzmir, it was revealed that the varieties of *Lavandula angustifolia* contained linalyl acetate at 52.84-54.58% (Karık et al., 2017). In another study conducted in our country, 48-59 compounds, constituting approximately 99.5-100.0% of *Lavandula angustifolia*'s essential oils, were characterized. The research, results determined linalool ranging from 31.9

to 50.0%, and linalyl acetate, from 15.4 to 42.0%, as the main components. In another study conducted in Romania, essential oils obtained from *Lavandula angustifolia* variety were analyzed qualitative and quantitative composition. Nine compounds were detected in different amounts, and two chemotypes were formed as Mailette and Vera varieties, linalool and linalyl acetate, respectively. Ecological differences in cultivation area explained differences in essential oil composition of Vera variety in linalyl acetate chemotype. In a similar study by Küçük et al. (2018), it was determined that lavender Mailette and Budakalaszi varieties contained the highest linalool ratios (52.9% and 47.0%), while 'Beate' variety contained the lowest (18.1%). This variety was the widest in terms of linalyl acetate (58.9%).

4. Conclusion

In this study, which was carried out to determine the high yield and quality lavender varieties that can adapt to the ecological conditions of Çorum province, lavander genotypes with different agricultural and vegetal characteristics were tested for the first time. It has been observed that there is no general problem in the species' adaptation to the region in general. It was determined that lavender variety gave satisfactory results in fresh stem flower yield, although slightly lower when compared to studies carried out in similar ecologies. On the other hand, it was determined that Vera and Silver varieties had the lowest potential in terms of fresh stem flower yield in both years. In addition, Raya and Munstead varieties stood out in terms of essential oil content. In this context, it is thought that the Sevtopolis variety Munstead and Sevtopolis varieties stand out both in terms of fresh stem flower yield and essential oil quality and recommended for the region.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Mustafa Akdogan: Validation, writing-original draft, methodology, investigation, conceptualization, formal analysis, data curation review and editing. **Serkan Uranbey**: Validation, formal analysis, review and editing. **Sinem Aslan Erdem**: Essential oil analysis, editing manuscript, proofreading.

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Data availability

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Consent for publication

Not applicable.

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Evaluation the UV sterilization of *Paenibacillus larvae* on beehive building materials

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ABSTRACT

This study presents the possibility of killing almost all microorganisms such as fungi, bacteria, spore forms, and viruses by sterilization process. European foulbrood (EFB) and American foulbrood (AFB) is a highly infectious bacterial honeybee disease caused by *Melissococcus plutonius* and *Paenibacillus larvae*, respectively. Removal of spores from contaminated beehives is a critical factor in controlling EFB and AFB. The purpose of this study was to evaluate the effectiveness of ultraviolet (UV) in killing *Paenibacillus larvae* spores on PVC, and wood hives. Hives infected with *Paenibacillus larvae* spores were treated with two UV powers (6 and 8 W) for up to 15 min. Sterilization at 8 W for 15 min resulted in a more than 6.6 log reduction in the number of *Paenibacillus larvae* spores on the PVC hives. Under the same experimental conditions, the reduction in wood hives was 6.2 log. Reductions achieved in *Paenibacillus larvae* spores on PVC hives after 5, 10 and 15 min of sterilization were significantly ($p<0.05$) higher than those on wood hives. So it is recommended to sterilize hives contaminated with spores with UV lamps.

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1. Introduction

Strong honey bee (*Apis mellifera*) colonies are needed for more production and effective pollination, but there is a relationship between disease factors and colony strength. Colonies are infected with fungi, viruses, and bacteria, such as European foulbrood (EFB) and American foulbrood (AFB), which is caused by spore-forming bacteria *Melissococcus plutonius* and *Paenibacillus larvae*, respectively (Alonso-Salces et al., 2017). If there is no cure for the disease, the spores will spread and infect the larvae, whereas spores do not cause infection in adult bees (Arredondo et al., 2017). AFB-affected larvae decompose to a glue-like colloid liquid, producing a specific smell (Fries et al., 2006). So, it causes economic loss to beekeepers. As it can live in colonies for many years, spreading via combs and honey products (Teixeira et al., 2018).

New Zealand legislation specifies that all bees, bee products, and appliances associated with AFB diseased colony must be burnt (Matheson and Reid, 2018). Generally, there are several methods of sterilizing beehives, such as flaming or scorching with a blowlamp (Gajger and Tomljanović, 2013) infrared; hand held electric paint stripper or immersion into molten paraffin (Del Hoyo et al., 1998); chemical sterilization with disinfectants or acetic acid; boiling in hot water or caustic soda (sodium hydroxide); steaming with hot air; Ozonation (Patil, 2014; Emrah and Kursat, 2018); low pressure plasma (Priehn et al., 2016); methyl oxide; and gamma radiation (De Guzman, 2011).

Options for sterilization available for PVC hives are limited compared to the range of treatments available for wooden hives (Fera, 2013). Del Hoyo et al. (1998) found that immersing wooden frames in molten paraffin was a very effective sterilization method, but handling of molten paraffin requires special equipment and protective clothing, which the small beekeeper does not have (Fera, 2013). *Paenibacillus larvae* spores are resistant to a wide variety of treatments such as heat, desiccation, and chemicals (Genersch, 2017). Dobbelaere et al. (2001) reported that the whole elimination of *Paenibacillus larvae* spores on wooden hives could be completed when heat and chemical disinfectants were used at high concentrations. They also reported that the scorching was not acceptable as it was effective against *Paenibacillus larvae* spores at the surface of the material. These disadvantages of the traditional sterilization methods for hive materials led to the use of alternative methods such methyl oxide, low pressure plasma, and gaseous ozone. However, these methods are expensive and available only to beekeepers who work near a treatment facility (James, 2011), so sterilization by ultraviolet (UV) is an effective process for killing spores. Ultraviolet (UV) is an electromagnetic wavelength that microorganisms absorb most of its energy resulting in a germicidal effect [photochemical reaction alters essential molecular components (DNA and RNA)]. Sterilization by ultraviolet (UV) dependent on duration and intensity (Newman and Bond, 2004). Also, the inactivation of viruses, protozoa, and bacterial pathogens by UV radiation has been stated by Chatzisymeon et al. (2011). They reported that UV radiation

aids oxidation to occur, which is known as photolysis processes and results in bond cleavage of organic molecules. Stewart-Wade (2011) stated also that, the UV light treatment is a chemical-free method which, uses exposure to a specific wavelength at a specific bulb power to inactivate microorganisms. UV light has radiation with a short wavelengths than visible light, and therefore has higher energy. UV radiation at 254 nm is the most common wavelength used for killing pathogens. Ultraviolet does not require storage, special handing or mixing considerations like chemical sanitizers. The PVC, and wood are the most common materials for constructing beehives, so the aim of this research was to find and evaluate a safe, effective, environmentally friendly and powerful sterilization method for all types of hives.

2. Materials and methods

2.1. Sample preparation

The source of spores forming bacteria *Paenibacillus larvae* caused by AFB was obtained from an infected apiary located 31º 10' 13" N, 31 47' 56" E at Meet-Salseel city, El-Dakahlia Governorate, Egypt. The spores of *Paenibacillus larvae* were used to contaminate the studied hive materials. Isolation, cultivation and bactericidal concentrations of AFB spores were carried out according to the method described by De Graaf et al. (2013). Cultures of *Paenibacillus larvae* were put in Petri dishes filled with agar and incubated at 35°C most of the cells speculated. Spore suspensions were achieved by transferring colonies from Petri dishes into distilled water and pooled. The suspension was centrifuged and suspended in distilled water. The spore concentration of the suspension was determined by plate count and adjusted to 107 spores/mL with distilled water. The suspension was saved in the refrigerator until usage. It was heated in a water base to activate spores before inoculation of the studied hive materials (Torlak and Isik, 2018). Pieces of hive materials (PVC, and wood) with dimensions of 5×4×2.5 cm were used. About 10 mL of the pooled spore suspension was sprayed on pieces of hive materials and saved in the refrigerator until Ultraviolet (UV) treatment.

2.2. Ultraviolet (UV) sterilization chamber

Two UV-C Lamps (Figure 1) with a wave length of 254 nm were used as a source of UV light. Its specifications are listed in Table 1.

Table 1. UV-C lamp specifications

Model	PHILIPS TUV 8W T5
Certification	CE, RoHS
Overall Length	302.5 (max) mm
Color temperature	Blue
Diameter	16 mm
Lamp current	0.15 A
Lamp voltage	56-265 V
Max. wattage	8 W
Average life (hrs)	9000

Pieces of hive materials (PVC, and wood) were placed in the sterilization chamber with the contaminate surface facing up and 45 cm away from the UV-C lamp. Each group of treatment included three pieces of each type of hive material. Contaminated pieces were subjected to two UV powers (6 and 8 W), which were valued by using the merged rheostat. Sterilization was performed three times (5, 10 and 15 min) at an ambient temperature of 26°C.

Figure 1. Ultraviolet sterilization chamber

2.3. Counting of Paenibacillus larvae

The number of *Paenibacillus larvae* on contaminated pieces before and after sterilization was recorded by colony counting technique, and the results were recorded as log cfu/piece.

2.4. Statistical analysis

Data were edited in MS Excel (Microsoft Corporation, Redmond, WA, USA). The Levene and Shapiro-Wilk tests were conducted in order to check for normality and homogeneity of variance (Razali and Wah, 2011). The data were statistically analyzed using the Costat Program (Oida, 1997) to determine the significant effect of the mentioned variables on the study based on the probability ($P<0.05$). The experiments were carried out three times in all. All graphs were drawn using Microsoft Excel 2016.

3. Results and discussion

Decreasing in mean count numbers of *Paenibacillus larvae* on sterilized PVC, and wood pieces after 5, 10, and 15 minutes and at UV power of 6 and 8 w are shown in Figures 2, and 3, respectively. The primary mean count numbers on PVC, and wood pieces were recorded as 6.8±0.2, and 6.6±0.3 log cfu/pieces, respectively. After 15 min of UV sterilization, spore count numbers on wood spices were significantly decreased ($p<0.05$) by 4.6 and 6.2 log cfu/pieces at UV power of 6 and 8 w, respectively. These decreases were nearly recorded on PVC pieces after only 10 minutes of UV sterilization. Decreases of 6.3 and 6.6 log cfu/pieces were recorded on PVC pieces after 15 min at UV power of 6 and 8 w, respectively. The results mentioned that decreases in count numbers of *Paenibacillus larvae* spores on PVC pieces after 10 and 15 min were significantly higher than those on wood pieces ($p<0.05$). This result may be attributed to the porous structure of wood. When materials have a porous surface, the spores can be embedded in cavities that prevent the interaction of UV Sterilizers with the spores, thus declining their potential for *Paenibacillus larvae* inactivation. As a result, the use of UV radiation has a significantly impact on surface porosity.

Ultraviolet radiation is an effective method for the inactivation of bacterial spores (Sanchez-Salas et al., 2017; Nyangaresi et al., 2019; Pendyala et al., 2019). In the present study, I used UV radiation to study the reduction in viability

of *Paenibacillus larvae* on hive building materials. However, it should be known that the UV effect on bacterial spores is dependent on duration and intensity (Newman and Bond, 2004). The effect of UV can be described by oxidative stress damage in spores (Taylor et al., 2020).

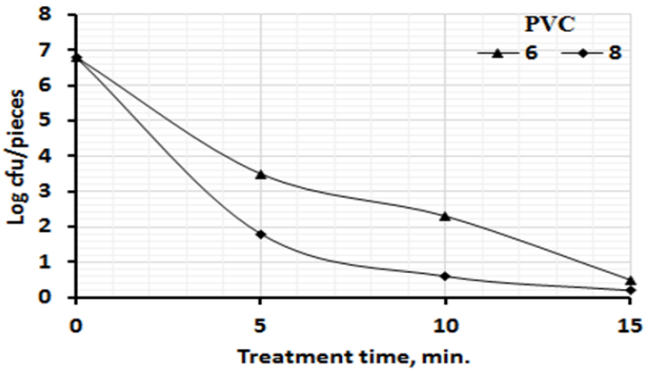

Figure 2. Population of *Paenibacillus larvae* on PVC pieces under different levels of UV power and treatment time

The best curve for the relationship between the population of *Paenibacillus larvae* "P" and treatment time "T" under both UV powers is the linear equation as shown in Equations (1 and 2).

At 6 w P = -0.402 T + 6.29 R^2 = 0.9561 → (1)
At 8 w P = -0.420 T + 5.50 R^2 = 0.7935 → (2)

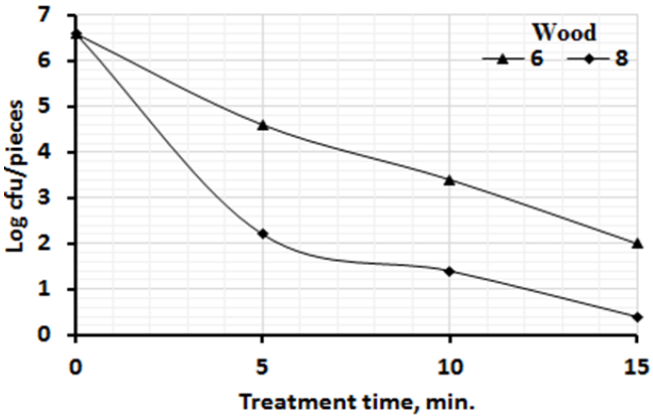

Figure 3. Population of *Paenibacillus larvae* on wood pieces under different levels of UV power and treatment time

At 6 w P = -0.300T + 6.40 R^2 = 0.9877 → (3)
At 8 w P = -0.388T + 5.56 R^2 = 0.8390 → (4)

Results in Table 2 indicated that the mean count numbers of *Paenibacillus larvae* decreased with beehive building materials, UV power and treatment time, according to the descending order (wood > PVC), (6 w >8 w) and (0 >5 > 10 > 15 min), respectively. Also, the building materials had a highly significant effect on the mean count numbers of *Paenibacillus larvae*. Minimum estimate is observed with PVC, respectively. Moreover, the different levels of UV power and treatment time affected the mean count numbers of *Paenibacillus larvae* significantly. Higher and lower estimates are shown at 6 and 8 w, respectively. Samples without treatment were coupled with the highest estimates compared to treatment times of 15 min which were coupled with the lowest estimates.

Table 2. Mean count numbers of *Paenibacillus larvae* affected by studied factors

Factors		Population, log cfu/pieces
Building materials	PVC	2.8±0.11a
	Wood	3.4±0.15b
	P-value	<0.0001
UV power	6 w	4.0±0.24a
	8w	2.9±0.12b
	P-value	<0. 0001
Treatment time	0 min.	6.7±0.31c
	5 min.	3.6±0.16a
	10 min.	2.3±0.11b
	15 min.	1.1±0.07d
	P-value	<0. 0001

4. Conclusion

This study investigated the inactivation performance of UV radiation toward *Paenibacillus larvae* causing AFB. Two building materials were studied under two UV power levels and three duration times. The minimum population of *Paenibacillus larvae* was obtained for the studied building materials after 15 min and at maximum UV power of 8 w. The reduction in the population of *Paenibacillus larvae* spores on PVC pieces was significantly higher than those on wood pieces. So, it is recommended to use UV radiation in the sterilization of beehive building materials, especially for PVC or plastic hives. Complete removal of *Paenibacillus larvae* that causes AFB from beehive building materials is an important requirement to avoid spread of American and foulbrood diseases. So, more studies are still necessary to achieve a more significant decrease in the population of *Paenibacillus larvae* on building hive materials.

Compliance with Ethical Standards

Conflict of Interest

The author declare that he has no conflict of interest.

Authors' Contributions

Mohamed Ali Ibrahim Al-Rajhi: Validation, writing-original draft, methodology, investigation, conceptualization, formal analysis, data curation.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

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