MASTER THESIS

In Order to Obtain the

PROFESSIONAL MASTER

in

BIODIVERSITY: CONSERVATION AND MANAGEMENT OF NATURAL RESOURCES

Presented and defended by:

Christy Agha

Title

Effect of applying LBM protocol to vineyards on grapes yield and quality

Supervisor
Dr. Charbel Mouawad

Reviewers
Dr. Fadi Dabaghi
Dr. Youssef Fakhry

Lebanese University-Faculty of sciences

ACKNOWLEDGMENTS

Firstly, I would like to show my deep gratitude to my supportive supervisor, Dr. Charbel Mouawad, for giving me the opportunity to work on this project, and for providing invaluable guidance through this research.

Further, I would like to thank Dr. Hassane Makhlouf, the head of the Biodiversity master, for guiding us through our master's degree, and for allowing me to work on his LBM protocol, and providing me with the detailed protocol in order to accomplish this project.

I would like to thank Mr. Ghassan Makhlouf for his generosity in allowing us to conduct this experiment in his fields and welcoming us to his land, while providing us with all the means to complete this experiment.

I am grateful to the members of the jury Dr. Fadi Dabaghi and Dr. Yousef Fakhry for their valuable time in reviewing my thesis.

I am grateful to all of those with whom I have had the pleasure to work during this and other related projects. I would like to show my appreciation to all the Doctors that have guided me through my academic years. Each of them has had their own impact on my personal and academic life, and I wouldn't have been where I am now without them. Thank you all for showing me what a good scientist should be.

Finally, I must express my very profound gratitude to my family and friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

ABSTRACT

This study was conducted on Chardonnay grapevines in order to evaluate the influence of the LBM protocol on their yield and quality, compared to the standard chemical treatment. The comparison of different parameters showed that the yield did not significantly differ between the two treatment; however, the vegetative growth and chemical composition of the grapes improved by the application of the LBM protocol.

Keywords: beneficial microorganisms; Chardonnay; biofertilizer; grapes quality; yield.

RESUME

Cette étude a été menée sur des vignes de Chardonnay afin d'évaluer l'influence du protocole LBM sur leur rendement et leur qualité, par rapport au traitement chimique standard. La comparaison des différents paramètres a montré que le rendement ne différait pas significativement entre les deux traitements ; cependant, la croissance végétative et la composition chimique des raisins ont été améliorées par l'application du protocole LBM.

Mots clés : microorganismes bénéfiques ; Chardonnay ; biofertilisant ; qualité des raisins ; rendement.

TABLE OF CONTENT

Contents

CHAPTER 0: INTRODUCTION

Winemaking is one of the world's most ancient practices, dating back thousands of years [1]. The Mediterranean region is amongst the first to begin the cultivation of grapes for wine making. This region has witnessed many civilizations such as the Phoenicians, Greeks and Romans who played a role in the evolution of grape cultivation and wine making [2]. Along the eastern shore of the Mediterranean Sea lies Lebanon, whose ideal climatic and geographic conditions have allowed the production of high-quality grapes. These properties characterize Lebanon as a respective terroir, suited for making fine wines that could compete with world class brands [3]. In addition to its traditional and social importance, viniculture plays a major role in the Lebanese economy, as it is ranked third among fruit crop production in the country [4]. Vineyards cover an area of 2636.85 ha, primarily distributed in the Beqaa valley, specifically in West Beqaa, North Beqaa and Central Beqaa, and to a lesser extent in North Lebanon, Mount Lebanon, South Lebanon and East Beqaa [3]. The 48 varieties that are planted in the Lebanese vineyards are mostly of excellent quality. The white grapes are distributed among 22 varieties [4].

One of the widely known noble grape wine variety of *Vitis vinifera* is the Chardonnay. It originated in France, and spread to most wine growing regions. Chardonnay is distinguished for producing superior table wines, as well as composing one of the finest sparkling wines, Champagne [5]. This variety begins blooming in the spring, and develops small compact clusters that change from yellow to amber when ripe [6]. Chardonnay is among the varieties that are mostly used by wineries in Lebanon, as it is used in almost 40 wineries and is ranked the fifth to be grown in Lebanese vineyards [3].

In recent years, the yield and quality of most wine grapes have been affected by climate change. Under those circumstances, farmers are compelled to use a wide

range of chemical fertilizers causing soil infertility, and leading to a higher incline in the yield and quality on the long term. Ergo, biofertilizer are considered promising alternatives to chemical fertilizers, especially in developing countries, where farmers tend to implement a solution based on its cost [7]. For these reasons, the LBM protocol was developed in order to rehabilitate Lebanese soils in a natural, safe and inexpensive manner. This was done by the isolation of beneficial bacteria and fungi from fertile Lebanese soils, and formulating a concentrated solution of beneficial microorganisms capable of degrading toxic substances, recycling minerals and making them available to plants.

This work explores the effect of applying the LBM protocol to *Vitis Vinifera* cv. Chardonnay on the grapes yield and quality. On this account, this research thesis is formed by three main parts. Initially, a literature review that handles the assessment of the mechanisms and parameters by which beneficial microorganism affect the yield and quality of chardonnay. The following part discusses the methodology adopted for data collection. The results are then discussed in the third part, where the interpretations of the different observations are elaborated.

CHAPTER I: STATE OF ART

1. Introduction

For ideal plant development, nutrients must be accessible in adequate and balanced amounts [8]. Beneficial microorganisms play a major role in plants growth, by stimulating beneficial interactions between biotic and abiotic factors. They increase nutrients availability [9], [10], protect plants from soil borne diseases [11]–[13], improve soil structure [14] and accelerate the decomposition of organic matter [15]. The increased stress caused by excessive use of chemical fertilizers and climate change has decreased viticulture productivity and has caused soil depletion [16]. Therefore, to keep up with the population growth while avoiding the depletion of the environment, a new trend of organic farming has been growing [17]. By substituting chemical fertilizers with biological fertilizers, farmers could ensure the sustainability of the soil while enhancing the diversity of beneficial fauna and flora [18]. Many studies have been done on the application of beneficial microorganisms to grapevines and the results were propitious [19]–[21].

2. LBM description

The exact composition of the LBM biofertilizer is kept confidential. However, three solutions have been used in the process of rehabilitation and fertilization of the grapevines. Soil activator, aerosol stabilizer and NFPC control, and soil regenerator. The description of the products and the guidelines of application have been provided by NESCO Lebanon, the manufacturing company, and are described as follows.

2.1 Soil activator

This organic compound is extracted from forest soil biota. It is constituted of nutrients and organic acids. It enhances symbiotic interactions between the soil and plant roots, which stimulates positive ion exchange processes between soil grains. This vital mechanism makes all the minerals available for the roots causing rapid growth of the roots and the vegetative complex of the plant. It has a key role in reactivating the vital metabolism of soils that have been degraded by the excessive use of chemical fertilizers. It disassembles these toxic molecules, stops their negative effect on the roots and contributes to their restoration.

2.2 Aerosol stabilizer and Nitrogen Fixator and Pest Control (NFPC)

The organic compound acts as a foliar organic fertilizer through enzymes specialized in absorbing air nitrogen, stabilizing it and inserting it into the plant through stomata leaves, thus ensuring a natural and sufficient nitrogen fertilization for all types of crops. This compound also contains microorganisms specialized in fungal disease control, as they destroy their cell walls, break them down and prevent them from reproducing on leaves and threatening the crop. If used according to the instructions, especially in terms of respecting spraying times, it can control various types of fungal diseases such as ophthalmia, spotting, blight, brown mold, black mold, fluffy whites and powdery mildew. In addition to having an effective role in preventing spiders from spreading to all types of crops and flowers and ornamental plants.

2.3 Soil regenerator

The soil regenerator is an organic compound that breaks down toxins and harmful minerals present in the soil. By doing so, it reinstates the vital interactions and reforms the physical, chemical and biological properties of the soil. The nutrients will then be available to the plants and the soil regains its fertility. It contains enzymes

that capture aerial nitrogen, ensuring natural nitrogen fertilization for all crops. It also stimulates the roots to absorb fertilizers, especially phosphorus and potassium, allowing the plants to have increased vegetative growth and number of buds and flowers.

3. Chardonnay main phenological stages according to the BBCH scale

Before the implementation of a new organic farming strategy, the growth cycle of the grapevine must be well understood. However, the phonology of the grapevine is influenced by environmental conditions such as temperature, humidity and solar radiation. These variables lead to changes in the chemical composition of grape maturation, by so affecting the growth stages [22]. The general BBCH-scale in an international coding system that covers the growth periods from the start of budburst to leaf fall. The phenology of the seasonal growth cycle of the grapevine is represented by two digits: the first digit of the scale refers to the principal growth stage, and the second digit refers to the secondary growth stage [23]. The principal stages and the main secondary stages are represented in (Table 1).

Table 1 Summary of the main phenological stages of Chardonnay according to the BBCH scale

BBCH code	Principal stage	BBCH code	Secondary stage	Time of year
1	Sprouting	01	Beginning of bud swelling	Beginning of April
		03	End of bud swelling	Beginning of April
		09	Bud burst	Mid-April

2	Leaf development	15	5 leaves unfolded	End of April
5	Inflorescence emergence	53	Inflorescence clearly visible	Mid-May
6	Flowering	61	Beginning of flowering (10% of flower hoods fallen)	Mid-May
		65	Beginning of flowering (50% of flower hoods fallen)	Mid-May
7	Development of fruits	71	Fruit set	End of May
		73	Berries groat-sized	End of May
		75	Berries pea-sized	Beginning of June
		79	Majority of berries touching	Mid-June
8	Ripening of berries	81	Beginning of ripening	Mid-July
		83	Berries developing color	Beginning of August
		89	Berries ripe for harvest	End of August
9	Senescence	93	Beginning of leaf fall	End of October

4. Parameters

The following parameters were adopted by many studies to evaluate the effect of beneficial microorganisms on the yield and quality of grapes [7], [21], [24]–[26]. The vegetative growth parameters include foliar area, vine height and width, number of secondary shoots per vine, number of new leaves per secondary shoot and mineral content in leaves (NPK). The yield in determined by counting the number of clusters

per vine, measuring the average weight of the cluster, calculating the average yield per vine, in addition to the dimensions of a single berry. As for the chemical composition of the grapes, the parameters are soluble solids content (SSC), total acidity, SSC / acidity ratio and alcohol potential. All the parameters should be obtained after the treatment is finished, at harvest time.

4.1. Foliar area

Leaf area is the calculation of canopy size, and is considered one of the determinants of the vine's health and status [27]. Leaf area provides crucial information for understanding the yield, quality and nutrient requirements of the grapevine [28]. Sufficient leaf area is necessary to supply the essential carbohydrates to ensure the growth of the vine in the current season and to store reserves for the following season [29]. Leaves keep growing until the beginning of the ripening of berries; therefore, measurements of the leaf area are usually done when the canopy has stopped growing [27]. Many methods have been established for measuring the vine leaves area. Some of these methods require the removal of the leaf sample which causes damage to the canopy, while others are non-destructive thus allowing the measurements to be repeated during the plant's growth period, thereby reducing the inconsistency related to destructive sampling procedures [30]. Many instruments are available in the market, including computer software [31], scanners [32], laser optics [33], digital image analysis [34]. These devises tend to be expensive, time-consuming and complex. Hence, a simpler and less expensive method is the estimation of the leaf area based on mathematical models using the length (L) and width (W) of the leaf [35]–[41].

Another method for the estimation of the canopy size is calculating the external surface of the vegetative cover by using parameters such as the height of vegetation measured from the base of the primary branch to its extremities, the thickness of the

vegetation on the row at mid-height, the distance between 2 rows, the total length of the row and the sum of the length of the holes in the row [42].

4.2. Vine height and width

The height and width of the vine are essential for calculating the vegetative cover. The length is measured from the head of the trunk till the tip of the secondary shoots. The width is measured from one side of the row to the other [42].

4.3. Number of secondary shoots per vine

The number of secondary shoots on the vine is one of the parameters to estimate the vigor of the vegetative cover [42].

4.4. Number of new leaves per secondary shoot

Counting the number of new leaves per secondary shoot is an assessment of the ability of the vine to invest in vegetative growth [42].

4.5. Mineral content in leaves (NPK)

In order to estimate the amount of nutrients absorbed by the plant, the mineral content of the leaves should be measured. The major nutrients are nitrogen, phosphorus and potassium [43]. Nitrogen plays an important role in plants metabolism. It is a component in proteins, enzymes, amino acids, polypeptides and many other biochemical compounds in the plant's system. It increases photosynthetic processes, leaf area and biomass; resulting in a higher crop yield [44]. Phosphorus is not as mobile as other macronutrients. It is an essential element for root development, stem strengthening, crop maturity, nitrogen fixation, biosynthesis and translocation of carbohydrates [45]. Potassium is vital to many plant processes. Its functions include enzymes activation, regulation of stomatal activity, ATP production, transportation of sugars, starch and protein synthesis, and enhancement of crop quality [46]. Many methods exist to measure the nutrients content in leaves. Nitrogen is usually measured by tissue analysis in a laboratory following the Kjeldahl Digestion

method [47]; however, this method is time consuming and destructive to the leaf sample. New in-field technologies have been developed for measuring the nitrogen content. These methods are based on the transmittance properties of leaves, leaf chlorophyll fluorescence, satellite imagery data, digital image processing, as well as canopy reflectance measurement systems [48]. Regarding measurement of the phosphorus and potassium content in leaves, it could be obtained either by the wet-digestion procedure or by the dry-ashing procedure. Both methods are adequate, but dry-ashing is safer, simpler and cheaper [49].

4.6. Number of clusters per vine

The number of clusters per vine should be recorded to help estimate the yield per vine [7], [26], [43], [50].

4.7. Average cluster weight

The weight of each individual cluster should be estimated in grams [7], [20], [26], [50].

4.8. Average yield per plant

The total yield per plant in kilograms by multiplying the average cluster weight by the number of clusters per vine [7], [20], [26], [50].

4.9. Berry dimensions

The diameter of a single berry should be recorded using a caliper[26].

4.10. Soluble solids content (SSC)

Sugars are the major soluble solids in grape juice. The sugar content of the grapes determines the wine quality, seeing that it regulates the percentage of alcohol in the wine. Many factors could influence the sugar content including changes in the environment and the viticulture management [51]. The most prevalent sugars are glucose and fructose [34]. An efficient way to measure the sugar content is by using a refractometer, it measures the refractive index which indicates how much a light

beam is bent when it passes through the fruit juice [52]. Another method to determine the content of reducing sugars in musts and wines is the Causse-Bonnans Fehling method [5].

4.11. Total acidity

Organic acids play a major role in determining the color, balance and taste of the wine; in addition to protecting the wine from bacteria and assuring the vitality of the yeast in the fermentation process [53].Total acidity is the combination of both volatile and fixed acidity, and it could be expressed in terms of tartaric, malic, citric, lactic, sulfuric, or acetic acid equivalents. Tartaric acid is the most significant indicator of grape vines acidity due to its high concentration and constant level through the whole ripening process [54]. The accurate measurement of tartaric acid is usually done by high-performance liquid chromatography [55]–[58]. Although total acidity could be calculated by performing titration against NaOH using phenolphthalein as an indicator [59].

4.12. SSC / acidity ratio

The soluble sugar content / acidity ratio is a predictors of grapes quality [60]. Good quality Chardonnay should have a ratio equal to or greater than 20 [61].

4.13. Alcohol potential

Alcohol potential is the total measurement of the alcohol that a wine may contain following fermentation. It is proportional to the level of sugar in the grapes and will enable the winemaker to determine the conversion rate of sugar into alcohol [5].

5. Conclusion

The purpose of this review was to view the trends and studies about using effective microorganisms as biofertilizers in wine grapes vineyards and to understand the methods that are commonly used to estimate the yield and quality of the grapes. The literature has revealed that most studies have taken place outside of Lebanon. Researchers have focused on table grapes quality and yield, and little was reviewed regarding wine grapes, especially Chardonnay. However, the methodology was helpful in understanding the techniques that are most effective.

CHARTER II: CONTRIBUTION

1. Experimental vineyard

This study was carries out from the end of April till the end of August of the same year (2020) to disclose the effect of applying LBM protocol to vineyards on grapes yield and quality. The experiment was conducted on 14-year-old uniform Chardonnay grapevines, in a private vineyard located in Hadath, Baalbek-Hermel Governorate, grown in a sandy clay soil, spaced 2.25 meters between rows and 1 meter within rows, under a drip irrigation system.

Thirty-two rows of Chardonnay were chosen for this study. The vines were split into two groups, each covering 16 rows (Figure 1). One group was treated according to the LBM protocol, and the other group was treated with chemical fertilizers. All the clusters were carefully harvested at the same time when they reached maturity in late August.

Figure 1 View of the study area (Google Earth image)

2. Experimental design

The implementation of two protocols took place on the same field (Figure 1.), planted with chardonnay grapevines having the same age and characteristics. All the vines were subjected to drip irrigation and the same weather conditions. The only variable was the type of protocol implemented, as the first 16 rows were subjected to the LBM protocol, and the remaining 16 rows were administered the chemical protocol. The following experiment was conducted in order to test many variables, from which we could estimate the grapevine's yield and quality as summarized in Table 2.

Table 2 Summary of the experimental design

	Modality 1	Modality 2
Protocol	LBM *	Chemical**
Grapevine type	Chardonnay	Chardonnay
Sample size	16 rows	16 rows
Irrigation	Drip irrigation	Drip irrigation
Tested variables	Cluster weight Number of clusters per vine Yield per vine % of diseases Dimensions of berries Number of new leaves per shoot Number of secondary shoots ESVC: External surface of vegetative cover	

*LBM: the vineyard was treated according to the LBM protocol which requires the administration of a soil activator, a soil regenerator and a nitrogen fixator and pest control.

**Chemical: the vineyard was treated with the conventional chemical protocol which involves the application of powder sulfur, wettable sulfur, fungicide and insecticide.

3. Treatments

3.1 Suggested LBM treatment

The LBM protocol is based on the application of three products which include a Soil Activator (SA), a Soil Regenerator (SR) and a Nitrogen Fixator and Pest Control product (NFPC). The exact treatment procedures of each product including the best period for its application, the optimal dose, the method of application and the frequency of administration are summarized in Table 3.

Table 3 Suggested LBM protocol

	LBM treatment protocol			
LBM product name	**Application period**	**Dose**	**Method of application**	**Frequency**
Soil Activator (SA)	Before bud burst	5 L SA /Dunum*	Mixing with water + drip irrigation	One time
Soil Regenerator (SR)	Before bud burst until harvest	1st treatment: 5 L SR / Dunum* Other treatments: 1 L SR / Dunum*	Mixing with water + drip irrigation	Every 15 days

Nitrogen Fixator and Pest Control product (NFPC)	Before bud burst until harvest	50 mL NFPC/20 L water	Mixing with water + spraying on leaves	Every week

*1 dunum = 1000 m^2

3.2 Applied treatments

However, due to adverse circumstances, the application of the LBM protocol was delayed till the end of April. Table 4 compares the differences between the suggested protocol and the protocol which was applied by the vineyard. The delay of the first application of Soil Activator has led to a setback in the entire procedure. In addition to the Soil Regenerator being applied for only 2 times in May, with an interval of 15 days. Furthermore, the Nitrogen Fixator and Pest Control was applied for 3 times every 10 days in May, even though the protocol suggests it be applied every week until harvest time (Table 3.). The reason behind this discontinuity in the protocol is the appearance of a fungal disease in June, identified as Powdery Mildew (Oidium), which results in leaf and fruit distortion, or even leaf and fruit drop in severe infections [5]. In this case, the winegrower decided to apply a chemical treatment in order to cease the infection.

Table 4 Comparison between the suggested LBM protocol and the LBM protocol applied by the vineyard

LBM product name	**Suggested LBM Protocol**	**Applied LBM Protocol by the vineyard**
Soil Activator (SA)	1st Application: March	1st Application: end of April
Soil Regenerator (SR)	1st Application: April Application time: repeat treatment every 15 days until harvesting time	1st Application: May Application time: 2 times treatment, once every 15 days
Nitrogen Fixator and Pest Control (NFPC)	1st Application: March Application time: repeat treatment every week until harvesting time	1st Application: May Application time: 3 times, once every 10 days

The chemical treatment was applied mid-June, when the infection had appeared to be spreading. Wettable sulfur, fungicides and insecticides were used to stop the infection. The application time if each treatment and the products administered are listed in Table 5.

Table 5 Comparison between the application of the LBM protocol and the chemical protocol

Application time	LBM protocol	Chemical protocol
April 2020	1st treatment: Soil Activator	1st treatment: Wettable Sulphur + insecticides 2nd treatment: Wettable Sulphur
May 2020	2nd treatment: Soil Regenerator 3rd treatment: Soil Regenerator 4th treatment: NFPC 5th treatment: NFPC	3rd treatment: Powder Sulphur 4th treatment: Wettable Sulphur + Insecticides + Fungicides 5th treatment: Powder Sulphur
June 2020	6th treatment: Wettable Sulphur 7th treatment: Wettable Sulphur + Insecticide + Fungicide	6th treatment: Powder Sulphur 7th treatment: Wettable Sulphur + Insecticide + Fungicide
July 2020	8th treatment: Wettable Sulphur + Insecticide + Fungicide	8th treatment: Wettable Sulphur + Insecticide + Fungicide

4. Measurements

4.1 Yield and physical characteristics of bunches

The following parameters were taken on the harvesting day which took place on August 26, 2020.

The vines chosen for the measurements were randomly propagated through the vineyard. Three samples of clusters were collected for each protocol, each sample constituting a bag weight about 1 kg. The average number of clusters per vines was recorded, and a generic cluster was chosen to be weighed using a small mechanical scale (1000g × 20g). The average cluster weight was multiplied by number of clusters/ vine and hence the average yield/ vine was calculated.

The dimensions of the berries (diameters) were measured using a Vernier caliper.

The number of infected clusters per vine was counted. This number was then divided by the number of clusters per vine in order to determine the percentage of infected clusters per vine.

4.2 Vegetative growth

The vegetative growth parameters were recorded mid-July, 2020.

The number of secondary shoots per vine was counted. In addition to the number of new leaves per secondary shoot.

The external surface of the vegetation cover represents the layer of foliage active for photosynthesis. It is used to calculate or estimate the ratio ESVC / Kg of grape at veraison and at maturity [42]. The formula is:

ESVC (m^2) = (2H + W) / E

- H: height of vegetation measured from the base of the primary branch to its extremities
- W: thickness of the vegetation on the row at mid-height
- E: distance between 2 rows

In a vineyard, there are usually missing vines and the vegetation hedge may be discontinuous. These missing values should be integrated in the calculation of SECV. A simplification consists in correcting the ESVC by multiplication with (1-T / D). Where:

- D: total length of the row
- T: sum of the length of the holes in the row

Therefore, the final formula would be:

$ESVC\ (m^2) = [\ (2H + W) / E] \times (1 – T/D)$

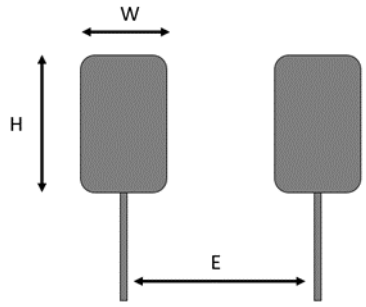

Figure 2 Illustration of the parameters fr calculating the external surface of the vegetative cover

4.3 Chemical characteristics of berries

Total acidity

Total acidity was measure in Chateau Kefraya winery's laboratory using the titration method.

A solution of sodium hydroxide NaOH (N/10) was prepared from 4g of sodium tablets dissolved in 1 liter of distilled water. To analyze the wine's acidity, a 5ml sample was measured with a pipette and poured into a 50ml beaker. A few drops of Bromothymol Blue Indicator were added and stirred with the sample. The solution of sodium hydroxide NaOH (N/10) was added to a burette and was slowly dropped into the beaker, while turning, until the color in the beaker changes to blue / dark green. The total acidity was then obtained based on the value observed on the burette.

Soluble sugar content (SSC)

Soluble sugar content was measured in Chateau Kefraya winery's laboratory.

Animal charcoal (almost 3 spatulas) were mixed with the wine sample. The solution was then poured over a funnel covered with filter paper, into volumetric flasks. Meanwhile, 5 mL of Fehling A solution and 5ml of Fehling B solution were poured in Erlenmeyer flasks using two different pipettes. The wine solution was poured in a burette and then the blue Fehling solution was brought to a boil. The wine solution was then poured, drop-by-drop, over the boiling Fehling solution until it turned to a brick red color. The X value on the burette was then noted, and the value of the reducing sugar in g/L was calculated by $\frac{25}{X}$.

SSC / Acidity ratio

The SSC / TA ratio was obtained from the quotient of the soluble sugar content and total acidity.

Alcohol potential

The alcohol potential of the wine sample was determined by its soluble sugar content. As 16.8 g/L of glucose produce 1° of alcohol, so the alcohol potential was calculated according to the following formula:

Alcohol potential = $\frac{16.8 \times 1}{X}$ (X: soluble sugar content in g/L)

5. Statistical analysis

The obtained data of this study were statistically analyzed according by Independent t-test on SPSS. Least Significant Difference (LSD) test was used to recognize the significance between the treatment means. A significance level of 5% was used for all statistical analyses.

CHAPTER III: RESULTS AND DISCUSSION

1. Yield and physical characteristics of bunches

The total yield of the Chardonnay grapevines was measured on the harvest day by the winegrower and the values were recorded as follows:

- LBM protocol: 5.3 tons / 16 rows
- Chemical treatment: 5.4 tons / 16 rows

The percentage difference between the two values is:

$$\frac{|Chemical\ yield\ -\ LBM\ yield\ |}{\frac{|Chemical\ yield + LBM\ yield|}{2}} \times 100 = \frac{|5.4 - 5.3\ |}{\frac{|5.4 + 5.3|}{2}} \times 100 = 1.87\%$$

A 1.87% decrease in the yield by application of the LBM protocol is considered non-significant.

On another note, the winegrower noticed a significant decrease in the yield between this year (2020) and the last (2019), reaching a substantial loss of 40% in the yield of the grapevines that were subjected to the chemical treatment. This decline could be caused by two reasons. One on which is due to climate change, as the Beqaa region faced an excessively hot weather where the temperature exceeded 40 ºC from the end of July till August of 2020, leading to grapes dehydration, reduction of juice and weight loss. The other reason is contributed to the adopted training method, seeing that the winegrower shifted from a single cordon training to a double cordon training.

Table 6 Effect of the LBM protocol and Chemical protocol on the yield parameters of the grapevines

Parameters	Groups	Effective	Mean ± SD	t test	p value
Cluster weight (g)	LBM	20	168.40 ± 14.41	-1.394	0.171^{ns}
	Chemical	20	178.15 ± 27.77		
Number of clusters per vine	LBM	20	48.55 ± 10.66	-1.869	0.069^{ns}
	Chemical	20	53.94 ± 7.29		
Average yield per vine (Kg)	LBM	20	8.15 ± 2.06	-1.973	0.056^{ns}
	Chemical	20	9.31 ± 1.64		
Dimensions of berries (mm)	LBM	20	11.76 ± 0.82	0.080	0.937^{ns}
	Chemical	20	11.75 ± 0.55		
% of disease per vine	LBM	20	5.09 ± 3.65	-18.179	0.000^{**}
	Chemical	20	22.86 ± 2.39		

*ns: Non significant; *: $p < 0.05$; **: $p < 0.01$*

As shown data in Table 4, it is clear that both LBM and chemical protocols gave non-significant differences between the means of the cluster weight, the number of clusters per vine, average yield per vine and the dimensions of the berries; as manifested by their p value being higher than 0.05. The means for LBM and chemical protocols were respectively, 168.40 ± 14.41 g and 178.15 ± 27.77g for the cluster weight, 48.55 ± 10.66 and 53.94 ± 7.29 for the number of clusters per vine, 8.15 ± 2.06 Kg and 9.31 ± 1.64 Kg for the average yield per vine and 11.76 ± 0.82 mm and 11.75 ± 0.55 mm for the dimensions of the berries. The results show that, despite the lack of chemical fertilizers, the grapevines subjected to the LBM protocol were able to match the yield and physical characteristics of the vines following the chemical protocol. This is mainly due to the microorganisms available in the LBM solutions, such as bacteria, yest and fungi, that increase the microbial diversity in the soil [62]. These microorganisms increase the availability of nutrients in the soil, as they are able to

absorb and translocate elements to the vine's roots; furthermore, they decompose complex organic substances and minerals in the soil and make them available to the plant [26]. Consequently, boosting the soil's fertility, leading to an increase the yield, growth and quality of the grapes. Moreover, the positive role of free nitrogen fixing bacteria is manifested in producing adequate amounts of growth regulators; such as auxin, which works with both ascorbic and citric acid, causing an enhancement in cell division and cell enlargement, which reflects positively on yield and physical characteristics of berries [7].

Regarding the percentage of disease in the vines, data from the same table clearly shows that the grapevines treated with the LBM protocol recorded a significantly lower percentage of disease spread among the clusters, as 5.09 ± 3.65 % of the clusters were infected. However, the vines treated with the chemical protocol reported a much higher percentage of infected clusters, as the average of clusters diagnosed with a disease reached 22.86 ± 2.39 % of the number of clusters per vine. The disease was identified as Oidium or powdery mildew which is caused by a fungus, Uncinula necator. This fungus grows distinctive extensions into living epidermal cells, causing the necrosis of the cell. Fungal growth is optimal between about 20 and 30 ºC. The infection can lead to leaf and fruit deformity, by covering the infected parts with a cottony covering of powdery mildew as shown in Figure 3, resulting in killing surface tissues before they reach maturity. Severe infection leads to leaf and fruit drop, as well as death of the shoot tip [63]. Early regulations must be taken in order to protect the fruits and minimize the damage to the foliage as it could affect the growth of the following year.

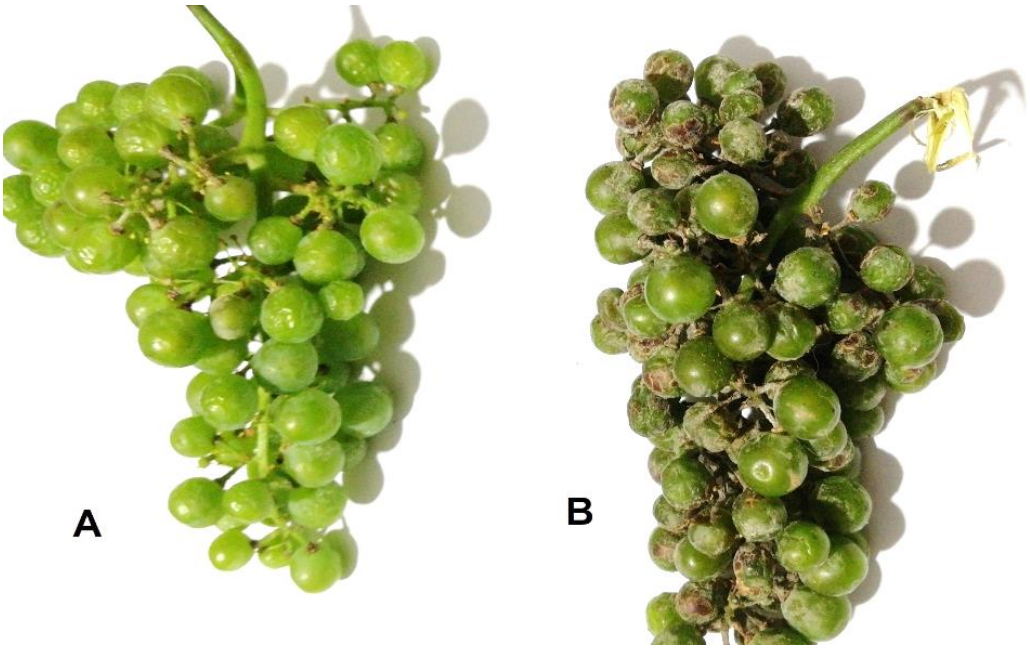

Figure 3 Comparison between a healthy cluster (A) and a cluster infected with powdery mildew (B)

Powdery mildew was spotted in May on the grapes subjected to the chemical protocol and they were immediately treated with wettable sulfur and fungicides. Wettable sulfur acts both as a preventive and curative agent. However, sulfur effectiveness is temperature dependent, it becomes much less active below 20 ºC, and dangerously phytotoxic above 30 ºC [5]. Unfortunately, shortly after the chemical treatment, a heat wave hit the area reaching temperatures as high as 45 ºC. This caused a sulfur burn on the grapes, which developed a hard, dark brown to black discoloration, leading to an asymmetrical berry development (Figure 4). The high temperatures limited the effectiveness of the sulfur, causing a high percentage of infected clusters per vine.

Figure 4 Cluster showing sulfur burns

Furthermore, powdery mildew was not spotted in the vines treated with the LBM protocol until June, meaning that the biofertilizer delayed the spread of the disease. This is mostly due to spraying the vines with the Nitrogen fixator and pest control (NFPC), which contains microorganisms specialized in fungal disease control. It acts by destroying the fungi's cell walls and breaking them down, thus preventing them from spreading and threatening the crops. Regardless, the disease was able to spread and infect the grapes. This could be attributable to the delay in the application of the LBM protocol, as it would have been optimal to start the treatment in March, whereas the treatment was initiated in May. In addition to the variation in the frequency of the treatment, as in was suggested to apply the NFPC product every week until harvest, though it was applied three times, once every 10 days (Table 4). This modification in the protocol allowed powdery mildew to spread across the vineyard. The farmer was then compelled to break the LBM protocol and to apply wettable sulfur on the grapevines in June (Table 5). After the termination of the infection, the farmer carried on with the chemical protocol until harvest since it would be ineffective to restart the LBM protocol at this stage.

2. Vegetative growth

Table 7 Effect of LBM protocol on the vegetative growth parameters of the grapevines

Parameters	Groups	Effective	Mean ± SD	t test	p value
Number of secondary shoots per vine	LBM	20	20 ± 9.84	3.525	0.000**
	Chemical	20	10.2 ± 4.17		
Number of new leaves per secondary shoot	LBM	20	20.75 ± 4.31	4.100	0.002**
	Chemical	20	17.05 ± 1.85		
External surface of vegetative cover (ESVC) (m^2)	LBM	20	0.9987 ± 0.064	5.389	0.000**
	Chemical	20	0.9082 ± 0.038		
ESVC (m^2) / Average yield per vine (Kg)	LBM	20	0.1299 ± 0.033	3.543	0.001**
	Chemical	20	0.1003 ± 0.017		

*ns: Non significant; *: $p < 0.05$; **: $p < 0.01$*

Table 7. shows that some vegetative growth traits outlined as the number of secondary shoots per vine, the number of new leaves per secondary shoot and the external surface of the vegetative cover were significantly improved by applying the LBM protocol. It is apparent that the mean of secondary shoots per vine almost doubled from 10.2 ± 4.17 when following the chemical protocol, to 20 ± 9.84 when applying the LBM protocol. As for the number of new leaves per secondary shoot, the LBM vines had a higher average of 20.75 ± 4.31 new leaves per secondary shoot compared to the chemical vines which have an average of 17.05 ± 1.85 new leaves per secondary shoot. This increase in the number of secondary shoots and the number of new leaves that formed on these shoots is manifested by the increase of the external surface of the vegetative cover as it was significantly higher in the vines subjected to the LBM protocol 0.9986 ± 0.064 m^2 than it was in the vines subjected to

the chemical protocol 0.9082 ± 0.038 m^2. The obtained results are compatible with those attained by various researchers who stated that beneficial microorganisms enhance the vegetative growth of some grape cultivars; Khalil, 2015 on Flame seedless grapevines; El-Mogy, 2017 on Crimson seedless grapevines; and Shaheen et al., 2013 on Superior grapevines. The improvement of the vine's vegetative growth by LBM may be attributed to the ability of some of these microorganisms to acquire essential nutrients from the soil, like nitrogen, magnesium and iron, which are key elements for chlorophyll formation. In addition to the effect of plant growth regulation substance produced by the effective microorganisms such as auxins, cytokines and gibberellins which affect production of root biomass and nutrient uptake [7]. Also, ascorbic and citric acid as antioxidants simulate growth and activate some physiological processes such as respiration and cell division and elongation which reflected positively on shoot length and leaf area [21].

Nonetheless, an excessive increase of vegetative growth could affect the sugar distribution dynamics, as a larger proportion of the assimilate would redirect towards the canopy rather than towards fruit development [64]. Essentially, it is vital to optimize carbon allocation to fruit sinks without disturbing growth and development in other parts of the grapevine, thus reducing the vegetative dominance without reducing the quality of the grapes. It is then critical to allow the leaves to function at their optimal efficiency, by creating a microclimate and physiological conditions that could allow maximum photosynthetic activity. An excess of canopy could disrupt the physiological balance in grapevines which is measured by vegetative growth, crop yield and grape composition [65]. Based on this, it is important to define a correct balance between the vegetative and productive relationship of the grapevine. The leaf-fruit ratio reflects the complex relationships between the source organs of the vine, which are the leaves and the sink organs, meaning the fruits. It is expressed in the amount of leaves needed to ripen 1 kg of grapes. It commonly varies from 0.5 to 2.5 m^2 of leaves per kg of grapes. Vines that fall within this range are considered well

balanced and capable of fully ripening their crop as well as producing high-quality wine [66]. But then again, the results in Table 7 illustrates that the ratio of ESVC (m2) per average yield per vine (Kg) is significantly different, 0.1299 ± 0.033 m^2 /kg for the LBM protocol and 0.1003 ± 0.017 m^2 /kg for the chemical treatment. These values do not fall within the range of a thriving vineyard. Meaning that the Chardonnay vines can bear to invest in their vegetative growth to produce berries of maximum soluble solids content, skin color, and total sugar accumulation in fruits. These results may reflect the attack of powdery mildew that led to the premature loss of canopy. This could be resolved by a number of solutions, apart from preventing powdery mildew, some of which are the alteration of the canopy training method, and the application of a larger dose of nitrogen fixing bacteria in order to boost photosynthesis [67].

To come to the point, the vegetative growth parameters measured for the chardonnay vines have a direct effect on the yield parameters. As the parameter for the external surface of the vegetative cover influences the percentage of disease per vine (Table 8).

Table 8 Correlation between the percentage of disease per vine and the external surface of the vegetative cover

Parameters	**Effective**	**Pearson correlation**	**P value**
% of disease per vine	20	-0.658	000*
External surface of vegetative cover (ESVC) (m^2)	20		

*ns: Non-significant; *: p < 0.05; **: p < 0.01*

The Pearson's r coefficient for the correlation, between the % of disease per vine and the external surface of the vegetative cover is -0.65, meaning that there is a moderate opposite relationship between the two variables. Accordingly, vines having a larger

external surface of vegetative cover tend to have less infections in their clusters (Figure 5).

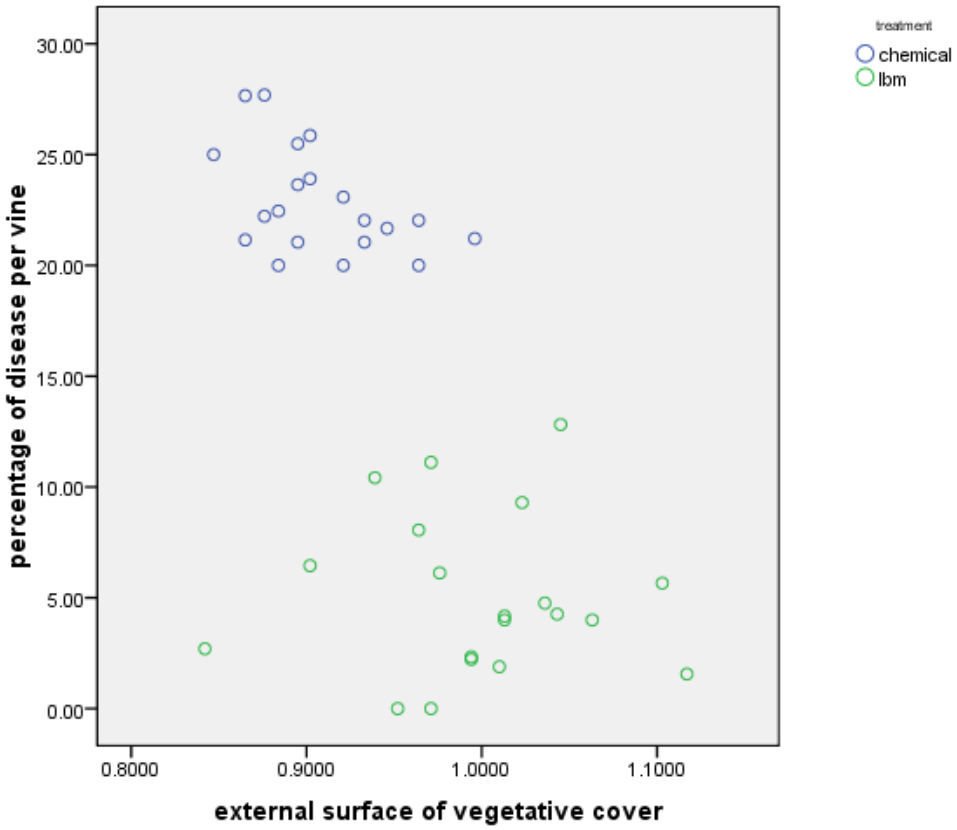

Figure 5 Visualization of the correlation between the percentage of disease per vine and the external surface of the vegetative cover

3. Chemical characteristics of grapes

Table 9 Effect of LBM protocol and chemical protocol on the chemical characteristics of grapes

	Sample number	**LBM treatment**	**Chemical treatment**
Total acidity (g/L H_2SO_4)	1	5.1	4.6
	2	5.2	4.6
	3	5.2	4.6
Soluble sugar content (g/L)	1	235.2	225.12
	2	228.48	228.48
	3	231.84	228.48
Soluble sugar content / Total acidity ratio	1	46.12	48.94
	2	43.94	49.67
	3	44.58	49.67
Alcohol potential (◦)	1	14	13.4
	2	13.6	13.6
	3	13.8	13.6

Data illustrated in Table 9 shows that the grapes subjected to the LBM treatment had a soluble sugar content and a total acidity that are higher than the grapes following the chemical treatment. This increase reflected in the same manner on the alcohol potential and the soluble sugar content / total acidity ratio. According to the standards of many wineries, the difference between both protocols is non-significant.

During the growth and ripening of the cluster, as the sugar accumulates in the fruit, the acid concentration decreases by the consumption of acids in plant respiration. The breathing phenomenon is influenced by temperature and rainfall, characterizing less acidic grapes in years of hot and dry summers. So, when high temperatures and

insolation are predominant, the vine metabolism favors a greater accumulation of soluble solids in the grape berries. Winemakers must harvest the grapes before acid levels fall too low, as the natural acids in the grape play an important role in the development of flavor and aroma compounds [61]. With this in mind, the LBM protocol, having higher values of total acidity, had the potential of delaying the harvesting time, thus giving more time for sugar accumulation and increasing the freshness of the wine.

CHAPTER IV: CONCLUSION AND FUTURE WORK

This research aimed to identify the effect that the LBM protocol will have on yield and quality of Chardonnay planted in western Beqaa, Lebanon. The experiment was conducted in order to compare the LBM treatment with the commonly used chemical treatment. Many researches have been done of the effect of beneficial microorganisms on the yield and quality of wine grapes, but none studied their effect on Chardonnay specifically in Lebanon's climate. Based on a quantitative analysis of different parameters, the Chardonnay grapevines subjected to the LBM protocol seemed to have improved in quality rather than the yield. As the data showed a non-significant difference between the yield of both treatments, but still the quality of the grapes enhanced and was manifested by a decrease in the percentage of disease in the clusters, a more vigor vegetative growth measured by the external surface of the vegetative cover per vine, and an slight increase in acidity and sugar content which positively affects the quality of the wine. The study experienced many challenges some of which were climatic, as heat waves struck the region; while others were due to pathogens identified as powdery mildew infecting the clusters and enticing the wine grower to interrupt the LBM protocol in the middle of June. Thus, the results obtained portrayed the effect of the LBM protocol form May till June as opposed to the suggested period from march till harvest. For this reason, additional studies must be done in order to obtain more exact results. For more accuracy, the LBM protocol must be applied for at least two consecutive years on the same vineyard, followed by a soil analysis to determine the dynamic of the soil after remediation with LBM.

To better understand the implications of these results, future studies could address the application of the LBM protocol on different grapevine varieties and at different phenological stages. In addition to its effect on fauna including pests and beneficial insects. And its ability to prevent or reduce other bacterial and fungal diseases

commonly found in vineyards. Further research could also be done on the economic difference between the LBM and chemical treatments, followed by a market study for the demand and consumption of organically grown wines in Lebanon.

As a final point, it became evident that Lebanon's diversity has enabled the production of a wide range of quality wines, all of which have not been immune to environmental and economic problems. Thus, the implementation of more sustainable viniculture applications is key for the continuity of the Lebanese terroirs.

BIBLIOGRAPHY

[1] L. Gorton, “in the Graduate School of The Ohio State University,” p. 331.

[2] C.-M. Bari, “TRADE AND LOGISTICS: THE CASE OF THE WINE INDUSTRY,” p. 19, 2014.

[3] R. Mohasseb and Y. Sassine, “STATE OF VINICULTURE IN LEBANON,” 2019, doi: 10.13140/RG.2.2.33847.57766.

[4] R. Mohasseb, Y. N. Sassine, Z. Sebaaly, L. Kfoury, and S. Kattar, “Survey study on the state of viniculture and wine production in Lebanon,” *Acta Hortic.*, no. 1276, pp. 15–22, Mar. 2020, doi: 10.17660/ActaHortic.2020.1276.3.

[5] R. S. Jackson, *Wine science: principles and applications*, 3rd ed. Amsterdam: Elsevier Acad. Press, 2008.

[6] L. J. Bettiga, *Wine grape varieties in California*, vol. 3419. UCANR Publications, 2003.

[7] H. A. Khalil, “The Potential of Biofertilizers to Improve Vegetative Growth, Nutritional Status, Yield and Fruit Quality of Flame Seedless Grapevines,” *Environ. Sci.*, p. 6, 2012.

[8] J.-H. Chen, “THE COMBINED USE OF CHEMICAL AND ORGANIC FERTILIZERS AND/OR BIOFERTILIZER FOR CROP GROWTH AND SOIL FERTILITY,” p. 11, 2006.

[9] A. A. A. El-Monem, M. M. S. Saleh, and E. A. M. Mostafa, “Minimizing the quantity of mineral nitrogen fertilizers on grapevine by using humic acid, organic and biofertilizers,” p. 5, 2008.

[10] A. E. RICHARDSON and R. J. SIMPSON, “Soil Microorganisms Mediating Phosphorus Availability: PHOSPHORUS PLANT PHYSIOLOGY,” *Plant physiology (Bethesda)*, vol. 156, no. 3, pp. 989–996, 2011.

[11] D. M. Weller, J. M. Raaijmakers, B. B. M. Gardener, and L. S. Thomashow, “Microbial populations responsible for specific soil suppressiveness to plant pathogens,” *Annual review of phytopathology*, vol. 40, no. 1, pp. 309–348, 2002.

[12] M. A. Hamad, S. A. Hussein, E. N. Mahmmoud, and A. M. Al-AAlim, “The inhibitory role of effective microorganisms on the growth of pathogenic bacteria,” *IJVS*, vol. 34, no. 1, pp. 153–158, Jan. 2020, doi: 10.33899/ijvs.2019.125653.1123.

[13] R. J. Cook, “Making Greater Use of Introduced Microorganisms for Biological Control of Plant Pathogens,” p. 28.

[14] C. Chenu, G. Stotzky, P. Huang, and J. Bollag, “Interactions between microorganisms and soil particles: an overview,” *Interactions between soil particles and microorganisms: Impact on the terrestrial ecosystem*, vol. 1, pp. 1–40, 2002.

[15] G. G. Brown, B. M. Doube, and C. A. Edwards, “Functional interactions between earthworms, microorganisms, organic matter, and plants,” *Earthworm ecology*, vol. 2, pp. 213–239, 2004.

[16] M. Maciejczak and J. Mikiciuk, “Climate change impact on viticulture in Poland,” *International Journal of Climate Change Strategies and Management*, 2019.

[17] H. Willer, M. Rohwedder, and E. Wynen, “Organic agriculture worldwide: current statistics,” *The world of organic agriculture. Statistics and emerging trends*, pp. 25–58, 2009.

[18] J. Döring, M. Frisch, S. Tittmann, M. Stoll, and R. Kauer, “Growth, Yield and Fruit Quality of Grapevines under Organic and Biodynamic Management,” *PLoS ONE*, vol. 10, no. 10, p. e0138445, Oct. 2015, doi: 10.1371/journal.pone.0138445.

[19] A. M. El-Salhy, K. I. A. Amen, A. A. B. Masoud, and A. E. Abozed, “Response of Ruby seedless and Red Roomy grapevines to application of some bio-fertilizers,” *Assiut J. Agric. Sci*, vol. 41, no. 5, pp. 125–142, 2011.

[20] M. A. G. Shaheen, S. M. Abdel-Wahab, and E. A. Hassan, “Effect of Some Soil Conditioners and Organic Fertilizers on Vegetative Growth and Quality of Crimson Seedless Grapevines,” p. 7, 2012.

[21] B. E. A. Belal, “Effect of some biostimulants of growth, yield and berry quality of King Ruby grapevines,” *Egyptian Journal of Horticulture*, vol. 42, no. 1, pp. 135–152, 2015.

[22] A. K. Parker, “Modelling phenology and maturation of the grapevine Vitis vinifera L.: varietal differences and the role of leaf area to fruit weight ratio manipulations,” p. 258.

[23] D. H. Lorenz, K. W. Eichhorn, H. Bleiholder, R. Klose, U. Meier, and E. Weber, “Phenological growth stages of the grapevine (Vitis vinifera L. ssp. vinifera)-Codes and descriptions according to the extended BBCH scale,” *Australian Journal of Grape and Wine Research*, vol. 1, no. 2, pp. 100–103, 1995.

[24] M. El-Boray, M. Mostafa, and D. Hamza, “EFFECT OF HUMIC ACID, BIO-FERTILIZERS AND MICRO-ELEMENTS ON LEAF MINERAL CONTENTS OF KING RUBY GRAPEVINES,” *Journal of Plant Production*, vol. 4, no. 6, pp. 871–883, Jun. 2013, doi: 10.21608/jpp.2013.73289.

[25] A.-R. M. A. Mohamed, F. H. Abdel-Aziz, M. A. Mohamed, and A. Gobara, “Effect of foliar application of sida compound fertilizer on growth, yield, and fruit chemical composition of ‘early superior’ grapevine,” *Journal of Horticultural Research*, vol. 21, no. 2, pp. 53–57, Dec. 2013, doi: 10.2478/johr-2013-0021.

[26] S. M. El-Mogy, “Effect of Arbuscular Mycorrhiza (AM) and effective micro-organisms (EM) on growth, yield and bunch quality of Crimson Seedless grapevines,” *Sciences*, vol. 7, no. 04, pp. 1005–1015, 2017.

[27] P. Skinkis, “Understanding Vine Balance,” p. 10.

[28] R. E. Smart, “Photosynthesis by grapevine canopies,” *Journal of Applied Ecology*, pp. 997–1006, 1974.

[29] E. W. Hellman, “Grapevine Structure and Function,” p. 16.

[30] P. Villanueva-Rey, I. Vázquez-Rowe, M. T. Moreira, and G. Feijoo, “Comparative life cycle assessment in the wine sector: biodynamic vs. conventional viticulture activities in NW Spain,” *Journal of Cleaner Production*, vol. 65, pp. 330–341, Feb. 2014, doi: 10.1016/j.jclepro.2013.08.026.

[31] M. Fladung and E. Ritter, “Plant leaf area measurements by personal computers,” *Journal of Agronomy and Crop Science*, vol. 166, no. 1, pp. 69–70, 1991.

[32] T. Tsonev and I. Sergiev, “Leaf area measurement using hand scanner,” p. 7.

[33] B. Baker, D. M. Olszyk, and D. Tingey, “Digital image analysis to estimate leaf area,” *Journal of Plant Physiology*, vol. 148, no. 5, pp. 530–535, 1996.

[34] Z. Li, C. Ji, and J. Liu, “LEAF AREA CALCULATING BASED ON DIGITAL,” p. 7.

[35] M. Weiss, F. Baret, G. J. Smith, I. Jonckheere, and P. Coppin, “Review of methods for in situ leaf area index (LAI) determination,” *Agricultural and Forest Meteorology*, vol. 121, no. 1–2, pp. 37–53, Jan. 2004, doi: 10.1016/j.agrformet.2003.08.001.

[36] E. A. Elsner and G. L. Jubb, “Leaf area estimation of Concord grape leaves from simple linear measurements,” *American Journal of Enology and Viticulture*, vol. 39, no. 1, pp. 95–97, 1988.

[37] J. T. Korva and G. A. Forbes, “A simple and low-cost method for leaf area measurement of detached leaves,” *Experimental Agriculture*, vol. 33, no. 01, pp. 65–72, 1997.

[38] S. Uzun and H. ÇeliK, “Leaf Area Prediction Models (Uzçelik-I) For Different Horticultural Plants,” p. 6.

[39] A. Gutierrez T. and A. Lavín A., “MEDICIONES LINEALES EN LA HOJA PARA LA ESTIMACIÓN NO DESTRUCTIVA DEL ÁREA FOLIAR EN VIDES cv. CHARDONNAY,” *Agric. Téc.*, vol. 60, no. 1, Jan. 2000, doi: 10.4067/S0365-28072000000100007.

[40] D. A. Grantz and L. E. Williams, “An Empirical Protocol for Indirect Measurement of Leaf Area Index in Grape (Vitis vinifera L.),” *HortSci*, vol. 28, no. 8, pp. 777–779, Aug. 1993, doi: 10.21273/HORTSCI.28.8.777.

[41] H.-Y. Lu, C.-T. Lu, M.-L. Wei, and L.-F. Chan, “Comparison of different models for nondestructive leaf area estimation in taro,” *Agronomy Journal*, vol. 96, no. 2, pp. 448–453, 2004.

[42] T. Dufourcq, “Adaptation de la conduite du vignoble : la gestion du rapport feuilles/fruits,” p. 4.

[43] M. A. Shaheen, S. M. A. ElWahab, F. M. El-Morsy, and A. S. S. Ahmed, “Effect of Organic and Bio-Fertilizers as a Partial Substitute for NPK Mineral Fertilizer on Vegetative Growth, Leaf Mineral Content, Yield and Fruit Quality of Superior Grapevine,” p. 9, 2013.

[44] S. J. Leghari *et al.*, “Role of Nitrogen for Plant Growth and Development: A Review,” p. 11, 2016.

[45] D. Thakur, R. Kaushal, and V. Shyam, “Phosphate solubilising microorganisms: role in phosphorus nutrition of crop plants-A review,” *Agri. Rev.*, vol. 35, no. 3, p. 159, 2014, doi: 10.5958/0976-0741.2014.00903.9.

[46] K. Prajapati and H. A. Modi, “THE IMPORTANCE OF POTASSIUM IN PLANT GROWTH – A REVIEW,” vol. 1, p. 11, 2012.

[47] D. W. Nelson and L. E. Sommers, “Total Nitrogen Analysis of Soil and Plant Tissues,” *Journal of AOAC INTERNATIONAL*, vol. 63, no. 4, pp. 770–778, Jul. 1980, doi: 10.1093/jaoac/63.4.770.

[48] R. Muñoz-Huerta, R. Guevara-Gonzalez, L. Contreras-Medina, I. Torres-Pacheco, J. Prado-Olivarez, and R. Ocampo-Velazquez, “A Review of Methods for Sensing the Nitrogen Status in Plants: Advantages, Disadvantages and Recent Advances,” *Sensors*, vol. 13, no. 8, pp. 10823–10843, Aug. 2013, doi: 10.3390/s130810823.

[49] G. Estefan, R. Sommer, and J. Ryan, “Methods of soil, plant, and water analysis,” *A manual for the West Asia and North Africa region*, vol. 3, 2013.

[50] A. Eman, A. El-Monem, M. Saleh, and E. Mostafa, “Minimizing the quantity of mineral nitrogen fertilizers on grapevine by using humic acid, organic and biofertilizers,” *Research Journal of Agriculture and Biological Sciences*, vol. 4, no. 1, pp. 46–50, 2008.

[51] A. Jordão, A. Vilela, and F. Cosme, “From Sugar of Grape to Alcohol of Wine: Sensorial Impact of Alcohol in Wine,” *Beverages*, vol. 1, no. 4, pp. 292–310, Nov. 2015, doi: 10.3390/beverages1040292.

[52] D. Garner, C. H. Crisosto, P. Wiley, and G. M. Crisosto, “Measurement of Soluble Solids Content,” p. 2.

[53] J. J. Ryan and J. A. Dupont, “Identification and analysis of the major acids from fruit juices and wines,” *Journal of Agricultural and Food Chemistry*, vol. 21, no. 1, pp. 45–49, 1973.

[54] J. Robinson and J. Harding, *The Oxford companion to wine*. American Chemical Society, 2015.

[55] E. G. Romero, G. S. Muñoz, P. M. Alvarez, and M. C. Ibáñez, “Determination of organic acids in grape musts, wines and vinegars by high-performance liquid chromatography,” *Journal of Chromatography A*, vol. 655, no. 1, pp. 111–117, 1993.

[56] O. Lamikanra, I. D. Inyang, and S. Leong, “Distribution and Effect of Grape Maturity on Organic Acid Content of Red Muscadine Grapes,” *J. Agric. Food Chem.*, vol. 43, no. 12, pp. 3026–3028, Dec. 1995, doi: 10.1021/jf00060a007.

[57] J. K. Palmer and D. M. List, “Determination of organic acids in foods by liquid chromatography,” *J. Agric. Food Chem.*, vol. 21, no. 5, pp. 903–906, May 1973, doi: 10.1021/jf60189a019.

[58] Y. Soyer, N. Koca, and F. Karadeniz, “Organic acid profile of Turkish white grapes and grape juices,” *Journal of Food Composition and Analysis*, vol. 16, no. 5, pp. 629–636, Oct. 2003, doi: 10.1016/S0889-1575(03)00065-6.

[59] W. Horwitz, P. Chichilo, and H. Reynolds, “Official methods of analysis of the Association of Official Analytical Chemists.,” *Official methods of analysis of the Association of Official Analytical Chemists.*, 1970.

[60] P. J. FELLERS, “The relationship between the ratio of degrees Brix to percent acid and sensory flavor in grapefruit juice,” *Food technology (Chicago)*, vol. 45, no. 7, pp. 68–75, 1991.

[61] A. Bender, V. B. Costa, V. Caliari, and M. B. Malgarim, “Maturation evolution of chardonnay grape for juice preparation,” in *BIO Web of Conferences*, 2016, vol. 7, p. 01004.

[62] T. Higa, “Effective Microorganisms: A New Dimension for Nature Farming,” p. 3.

[63] F. Halleen and G. Holz, “An Overview of the Biology, Epidemiology and Control of Uncinula necator (Powdery Mildew) on Grapevine, with Ref ere nee to South Africa,” *South african journal of Enology and Viticulture*, vol. 22, no. 2, pp. 111–121, 2001.

[64] J. J. Hunter, “Implications of seasonal canopy management and growth compensation in grapevine,” *South African Journal of Enology and Viticulture*, vol. 21, no. 2, pp. 81–91, 2000.

[65] J. Hunter and J. H. Visser, “The Effect of Partial Defoliation on Growth Characteristics of Vitis vinifera L. cv. Cabernet Sauvignon II. Reproductive Growth,” *S. Afr. J. Enol. Vitic*, vol. 11, no. 1, p. 7, 1990.

[66] W. M. Kliewer and N. K. Dokoozlian, “Leaf Area/Crop Weight Ratios of Grapevines: Influence on Fruit Composition and Wine Quality,” p. 12, 2005.

[67] N. Vaillant-Gaveau *et al.*, “Relationships between carbohydrates and reproductive development in chardonnay grapevine: impact of defoliation and fruit

removal treatments during four successive growing seasons," *OENO One*, vol. 48, no. 4, p. 219, Dec. 2014, doi: 10.20870/oeno-one.2014.48.4.1694.

LIST OF ABREVIATIONS

LBM: Lebanese beneficial microorganisms

NFPC: Nitrogen fixator and pest control

NESCO: National environmental solution company

SA: Soil activator

SR: Soil regenerator

SSC: Soluble solids content

TA: total acidity

ESVC: External surface of vegetative cover

g: grams

Kg: kilograms

H: height of vegetation measured from the base of the primary branch to its extremities

W: thickness of the vegetation on the row at mid-height

E: distance between 2 rows

D: total length of the row

T: sum of the length of the holes in the row

mL: milliliters

g/L: grams per litter

SPSS: Statistical Package for the Social Sciences

LSD: Least significant difference

mm: millimeters

m^2: meters squared

m^2 /kg: meters squared per kilograms

LIST OF SYMBOLS

ºC: Degrees Celsius

%: Percent

LIST OF FIGURES

LIST OF TABLES

ANNEX

1. ANNEX 1: LBM PARAMETERS DATA

row number	number of clusters	Berries Dimensions (mm)	Cluster Weight (g)	Average yield per vine (g)
5	36	11.58	165	5940
5	37	12.58	155	5735
5	42	12.40	175	7350
5	27	11.83	158	4266
7	45	12.15	135	6075
7	43	12.13	150	6450
7	50	12.15	170	8500
7	48	11.08	180	8640
9	62	10.90	195	12090
9	64	11.15	190	12160
9	50	10.45	170	8500
9	53	10.50	165	8745
13	39	13.10	185	7215
13	49	11.93	165	8085
13	62	11.68	160	9920
13	62	12.73	180	11160
15	53	10.37	165	8745
15	49	11.79	155	7595
15	47	13.07	180	8460
15	43	11.75	170	7310

Average yield per vine (kg)	average height (m)	average width (m)	(2H+W)/E (m^2)	ESVC (m^2)
5.94	1.25	0.5	1.3333	0.9714
5.735	1.05	0.5	1.1556	0.8419
7.35	1.35	0.5	1.4222	1.0362
4.266	1.25	0.5	1.3333	0.9714
6.075	1.20	0.5	1.2889	0.9943
6.45	1.20	0.5	1.2889	0.9943
8.5	1.30	0.5	1.3778	1.0629
8.64	1.12	0.5	1.2178	0.9394
12.09	1.20	0.5	1.2889	1.0127
12.16	1.35	0.5	1.4222	1.1175
8.5	1.20	0.5	1.2889	1.0127
8.745	1.33	0.5	1.4044	1.1035
7.215	1.43	0.5	1.4933	1.0453
8.085	1.28	0.5	1.3600	0.9520
9.92	1.20	0.5	1.2889	0.9022
11.16	1.30	0.5	1.3778	0.9644
8.745	1.25	0.5	1.3333	1.0095
7.595	1.20	0.5	1.2889	0.9759
8.46	1.30	0.5	1.3778	1.0432
7.31	1.27	0.5	1.3511	1.0230

ESVC (m^2) / Average yield per vine (kg)	number of secondary shoots	average number of leaves per shoot	disease	% disease
0.1635	43	21	4	11.11
0.1468	12	24	1	2.70
0.1410	31	21	2	4.76
0.2277	12	21	0	0.00
0.1637	10	25	1	2.22
0.1542	26	23	1	2.33
0.1250	12	20	2	4.00
0.1087	22	25	5	10.42
0.0838	18	25	3	4.84
0.0919	4	13	1	1.56
0.1191	6	14	2	4.00
0.1262	18	18	3	5.66
0.1449	31	18	5	12.82
0.1177	30	31	0	0.00
0.0909	22	15	4	6.45
0.0864	23	19	5	8.06
0.1154	25	22	1	1.89
0.1285	17	23	3	6.12
0.1233	11	17	2	4.26
0.1399	27	20	4	9.30

2. ANNEX 2: CHEMICAL PARAMETERS DATA

row number	number of clusters	Berries Dimensions (mm)	Cluster Weight (g)	Average yield per vine (g)
18	54	11.55	160	8640
18	47	11.875	185	8695
18	40	11.875	180	7200
18	65	10.95	135	8775
21	57	12.75	210	11970
21	60	12.325	200	12000
21	49	11.625	190	9310
21	60	11.425	120	7200
25	55	11.85	160	8800
25	46	12.25	155	7130
25	59	13.025	200	11800
25	50	11.45	160	8000
29	58	11.05	170	9860
29	39	11.125	180	7020
29	59	11.275	180	10620
29	58	11.575	185	10730
31	51	11.56	165	8415
31	57	12.03	193	11001
31	52	12.14	170	8840
31	55	11.26	185	10175

Average yield per vine (kg)	average height (m)	average width (m)	(2H+W)/E (m^2)	ESVC (m^2)
8.64	1.25	0.5	1.3333	0.8762
8.695	1.23	0.5	1.3156	0.8645
7.2	1.20	0.5	1.2889	0.8470
8.775	1.25	0.5	1.3333	0.8762
11.97	1.25	0.5	1.3333	0.9333
12	1.27	0.5	1.3511	0.9458
9.31	1.17	0.5	1.2622	0.8836
7.2	1.30	0.5	1.3778	0.9644
8.8	1.23	0.5	1.3156	0.9209
7.13	1.20	0.5	1.2889	0.9022
11.8	1.30	0.5	1.3778	0.9644
8	1.17	0.5	1.2622	0.8836
9.86	1.35	0.5	1.4222	0.9956
7.02	1.23	0.5	1.3156	0.9209
10.62	1.25	0.5	1.3333	0.9333
10.73	1.20	0.5	1.2889	0.9022
8.415	1.25	0.5	1.3333	0.8952
11.001	1.25	0.5	1.3333	0.8952
8.84	1.20	0.5	1.2889	0.8654
10.175	1.25	0.5	1.3333	0.8952

ESVC (m^2) / Average yield per vine (kg)	number of secondary shoots	average number of leaves per shoot	disease	% disease
0.1014	12	17	12	22.22
0.0994	7	15	13	27.66
0.1176	5	14	10	25.00
0.0999	15	20	18	27.69
0.0780	6	18	12	21.05
0.0788	9	20	13	21.67
0.0949	6	17	11	22.45
0.1340	21	16	12	20.00
0.1046	9	16	11	20.00
0.1265	13	19	11	23.91
0.0817	7	18	13	22.03
0.1104	16	17	10	20.00
0.1010	15	14	14	24.14
0.1312	7	15	9	23.08
0.0879	6	18	13	22.03
0.0841	8	20	15	25.86
0.1064	10	16	13	25.49
0.0814	11	18	12	21.05
0.0979	12	17	11	21.15
0.0880	9	16	13	23.64

3. ANNEX 3: SPSS DATA

Cluster weight

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
cluster weight (g)	lbm	20	1.6840E2	14.40541	3.22115
	chemical	20	1.7815E2	27.76836	6.20919

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
cluster weight (g)	Equal variances assumed	3.727	.061	-1.394	38	.171	-9.75000	6.99499	-23.91061	4.41061
	Equal variances not assumed			-1.394	28.536	.174	-9.75000	6.99499	-24.06647	4.56647

Number of clusters per vine

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
cluster weight (g)	lbm	20	1.6840E2	14.40541	3.22115
	chemical	20	1.7815E2	27.76836	6.20919

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
cluster weight (g)	Equal variances assumed	3.727	.061	-1.394	38	.171	-9.75000	6.99499	-23.91061	4.41061
	Equal variances not assumed			-1.394	28.536	.174	-9.75000	6.99499	-24.06647	4.56647

Average yield per vine (Kg)

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
cluster weight (g)	lbm	20	1.6840E2	14.40541	3.22115
	chemical	20	1.7815E2	27.76836	6.20919

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
cluster weight (g)	Equal variances assumed	3.727	.061	-1.394	38	.171	-9.75000	6.99499	-23.91061	4.41061
	Equal variances not assumed			-1.394	28.536	.174	-9.75000	6.99499	-24.06647	4.56647

Dimensions of a berry

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
dimensions of a berry	lbm	20	11.7660	.82266	.18395
	chemical	20	11.7482	.55166	.12336

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
dimensions of a berry	Equal variances assumed	2.447	.126	.080	38	.937	.01775	.22148	-.43062	.46612
	Equal variances not assumed			.080	33.214	.937	.01775	.22148	-.43275	.46825

Percentage of disease per vine

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
percentage of disease per vine	lbm	20	5.0915	3.65835	.81803
	chemical	20	22.8595	2.39225	.53492

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
percentage of disease per vine	Equal variances assumed	3.098	.086	-18.179	38	.000	-17.76800	.97740	-19.74665	-15.78935
	Equal variances not assumed			-18.179	32.737	.000	-17.76800	.97740	-19.75715	-15.77885

Number of secondary shoots

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
number of secondary shoots	lbm	20	20.0000	9.84084	2.20048
	chemical	20	10.2000	4.17511	.93358

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
number of secondary shoots	Equal variances assumed	12.352	.001	4.100	38	.000	9.80000	2.39033	4.96103	14.63897
	Equal variances not assumed			4.100	25.625	.000	9.80000	2.39033	4.88310	14.71690

Number of new leaves per secondary shoot

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
average number of leaves per shoot	lbm	20	20.7500	4.31491	.96484
	chemical	20	17.0500	1.84890	.41343

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
average number of leaves per shoot	Equal variances assumed	7.748	.008	3.525	38	.001	3.70000	1.04969	1.57502	5.82498
	Equal variances not assumed			3.525	25.749	.002	3.70000	1.04969	1.54132	5.85868

External surface of vegetative cover

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
external surface of vegetative cover	lbm	20	.998550	.0643105	.0143803
	chemical	20	.908200	.0385563	.0086215

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
external surface of vegetative cover	Equal variances assumed	2.611	.114	5.389	38	.000	.0903500	.0167667	.0564076	.1242924
	Equal variances not assumed			5.389	31.096	.000	.0903500	.0167667	.0561584	.1245416

ESVC / Average yield per vine

Group Statistics

	treatment	N	Mean	Std. Deviation	Std. Error Mean
ratio	lbm	20	.129930	.0333651	.0074607
	chemical	20	.100255	.0170165	.0038050

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
ratio	Equal variances assumed	4.107	.050	3.543	38	.001	.0296750	.0083749	.0127208	.0466292
	Equal variances not assumed			3.543	28.258	.001	.0296750	.0083749	.0125268	.0468232

Correlation between the percentage of disease and the external surface of the vegetative cover

Correlations

		percentage of disease per vine	external surface of vegetative cover
percentage of disease per vine	Pearson Correlation	1	-.658**
	Sig. (2-tailed)		.000
	N	40	40
external surface of vegetative cover	Pearson Correlation	-.658**	1
	Sig. (2-tailed)	.000	
	N	40	40

**. Correlation is significant at the 0.01 level (2-tailed).