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ANALYSIS OF IMPACT OF CALCIUM FOLIAR FEED ON THE WILTING RATE OF ROSE FLOWERS OVER TIME

By

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Declaration

I, DUSHIMIRIMANA Justine, hereby declare that the work presented in this thesis is my own work. It has not been previously presented or submitted for any comparable academic award. Except where stated otherwise by reference or acknowledgment, the work presented is entirely my own.

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Dedication

I dedicate this work to my husband Jean Marie Vianney and to all of our children: INEZA MUCYO Darlin and INEZAYE JABO Elio, and my parents.

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DUSHIMIRIMANA Justine

Abstract

Rose flower contributes to Rwanda's economy and it is distributed along the world-wide as it is a commercial crop. The quality of rose flower also contributes to the development of the export market. Despite this contribution, profitable production of rose flower is limited by the wilting process over time which lead to the lower production. A study was set up to analyze if the Calcium foliar feed can reduce the wilting rate of rose flowers across the time. The main objective of this thesis is to analyze the impact of Calcium foliar feed on the wilting rate of rose flowers over time using growth curve model. The data was analyzed using MATLAB R2014a and the results show that the fitted growth curves or trends in all groups is modeled with a second order polynomial. The results also reveal that the foliar feed 5 produces the highest average wilting score while foliar feed 2 has the least average score. The results also show that the wilting rate of rose flowers in all groups increase as time of measurements increase. In addition, the results show that the wilting rate of rose flowers are not the same in all foliar feeds over time. Moreover, foliar feed has significant effects on the wilting process of rose flowers over time.

Contents

Chapter 1

INTRODUCTION

1.1 Background

The agricultural sector is a large contributor to Rwanda's national economy. A significant share of this contribution is coming from the horticultural sector. The Government of Rwanda has a strong focus on increasing horticultural production and is simultaneously supporting the development of the export market. According to data from the Ministry of Agriculture and Animal resources, Rwanda's horticulture generates $ 5 million in 2005 and continues to increase to $ 25 million in 2018 [1]. Evaluation of Rwanda's horticultural exports in 2018 revealed that vegetables, fruits, and flowers generated $ 12.9 million, $ 7.8 million and $ 4.1 million respectively to the total earnings in this sector [1]. Hence, the rose flowers are the one among the flowers that generate this income. The rose is one of the oldest flowers in cultivation and is still considered as one of the most popular garden flowers today [2]. Rose flowers contribute to the poverty reduction, quality of education in Rwanda, etc. Rose cut flower production contributes significantly to Rwanda's economy and its quality is therefore paramount for sustainability on the international export markets [3]. Even if its contribution is significant, there are several factors which can affect the patterns of change of the wilting scores of leaves or flowers over time and this change leads to the lower production of rose flower. Those factors are the covering materials, foliar feeds, climate change, etc. Wilting is the loss of rigidity of non-woody parts of plants. This occurs when the turgor pressure in non-lignified plant cells falls towards zero, as a result of diminished water in the cells. Wilting also serves to reduce water loss, as it makes the leaves expose less surface area [4]. The rate of loss of water from the plant is greater than the absorption of water in the plant. Wilting diminishes the ability of the plant to grow and transpire and in some cases; it may lead to the death of the affected plant. This work is based on an agricultural split-split plot experiment with 3 experimental factors: covering material (3 levels), rose flower variety (2 levels) and foliar feeds (5 levels), each with different concentration levels of calcium. The experiment was set up in 3 plots (main plots) contained in block factor (3 levels). Each of the main plots was split into 2 sub plots (Split-plots) and each of these sub plots was split into 5 sub-sub plots (Split-Split-plots). Each type of the covering material was systematically assigned in each of the main plots, each level of the plant variety was systematically assigned to the sub plots and each of the foliar feed was systematically assigned to the sub-sub plots. Therefore, the foliar feeds were contained in variety and the varieties were contained in the covering materials. The entire experiment was conducted in a different time period with the same runs and the results recorded. The wilting scores are taken at 4 different time points (i.e days 0,3,6 and 9). This experiment was done by International Centre for Tropical Agriculture (CIAT) Rwanda [5]. It will be interested in one factor which foliar feed with five treatment groups and only one variety of rose flowers. So, there are many methods used in agricultural research. One of the methods used in this research is the Growth Curve Model (GCM) for analyzing longitudinal data. The main aim of this study is to apply this model to analyze the effects of Calcium foliar feed on the wilting rate of rose flowers over time.

1.2 Problem statement

Rose flower contributes to Rwanda's economy as it is a commercial and ornamental crop. Even if this contribution is significant, rose flower is facing with several challenges. One of them is the wilting process of it across the time. Calcium, one of the most essential elements of the cell wall that plays an important role in flower's life is not readily available to plants because of its low mobility and translocation. In addition, Calcium foliar feed can affect the wilting process of rose flowers over time. This change leads to the lower production of rose flower. The problem is to identify the trends of growth curve for all different concentration of Calcium foliar feeds over time and to investigate whether the wilting rate of rose flowers will be the same or not in all foliar feeds across the time. The Growth curve model will be formulated to analyze the impact Calcium foliar feed on the wilting rate of rose flowers over time.

1.3 Objectives

1.3.1 General objective

The main objective of this research is to apply growth curve model to analyze the effects of Calcium foliar feed on the wilting rate of rose flowers over time

1.3.2 Specific objectives

The specific objectives are the following:

1. To estimate the trends of wilting rate of rose flowers in all treatment groups.
2. To test whether the estimated trends or curves are significant or not for the GCM.
3. To test if the wilting rate of rose flowers will be the same or not across the time in the 5 different concentration of Calcium foliar feeds.

1.4 Significance of the study

Agricultural sector is one of the areas which have attracted a lot of interest from researchers. This shows that the amount of data being produced and handled by this sector has been increased. Because of this, to develop more advanced and statistical tools are needed, which ensure that scientists get the appropriate results from the available data. Experiments with plants are realized in fields and greenhouses. In most cases, we observe that such experiments require the split- plot or split-split plot experimental designs described in Section 2.4.3 and 2.4.4. Analysis of split-split plot data based on the time of measurements gives longitudinal or repeated data. It requires the use of statistical data analysis such as MANOVA and GCM. This research explains the theory behind the GCM and describes how this model can be used to analyze the impact of Calcium foliar feed on the wilting rate of rose flowers over time in the split-split plot experiment. Also, this study proposes the Likelihood Ratio Test (LRT) statistic for testing general linear test of hypothesis in the GCM. Therefore, the information and results of this research are important because they give the readers, researchers and scientists with limited statistical background a way of accurately applying GCM, analyzing the longitudinal data and appropriately interpreting the results obtained. The users of the research results are also the agriculture researcher and CIAT (International Centre for Tropical Agriculture) Rwanda in order to identify suitable concentration of Calcium feed that will assist in getting high quality production of rose flowers.

1.5 Definitions and notations

1.5.1 Definitions

- **Wilting** is the loss of rigidity of non-woody parts of plants in which the rate of loss of water from the plant is greater than the absorption of water in the plant.
- **Foliar feed** is a substance containing nutrients that is applied to the leaves or flowers of a plant.
- **Treatment** is the number of levels in a factor.
- **Longitudinal data**, sometimes called panel data, are data that are collected through series of repeated observations of the same subjects over some extended time frame and are useful for measuring change. They consist of observations (i.e., measurements) taken repeatedly through time on a sample of experimental units (i.e. individuals, subjects).
- In statistics, a **design matrix**, also known as model matrix or regressor matrix, is a matrix of values of explanatory or variables of a set of objects. Each row represents an individual object, with the successive columns corresponding to the variables and their specific values for that object.The design matrix contains data on the independent variables (also called explanatory variables) in statistical models which attempt to explain observed data on a response variable (often called a dependent variable) in terms of the explanatory variables [6].

1.5.2 List of notations

Throughout this thesis, the matrices will be denoted with bold font uppercase letters, vectors with bold font lowercase letters, scalars and elements in matrices and vectors with lowercase letters.

$r(\bullet)$: rank of a matrix

$\mathbf{A}^{'}$: transposed matrix

$\mathbf{I}$: identity matrix

$|\mathbf{A}|$: determinant of $\mathbf{A}$

$\mathbf{K}_{p,q}$: commutation matrix

$\frac{\partial \mathbf{Y}}{\partial \mathbf{X}}$: partial derivative of matrix

$N_{n,p}(\bullet, \bullet, \bullet)$: matrix normal distribution,

$W_{r(\mathbf{M})}(\bullet, \bullet)$: Wishart distribution,

$\chi^2_{p_0 m}$: Chi-square distribution with $p_0 m$ degrees of freedom,

$C(\mathbf{A})$: column space

$\mathbf{X}$: random matrix,

$P_{\mathbf{X}}$: is a projection matrix on the range space of $\mathbf{X}$,

$\mathbf{P}^{'}_{\widehat{\mathbf{Q}}^{'}_1, \mathbf{A}_2, \widehat{\mathbf{S}}_2}$: is a projection matrix on the range space of $\widehat{\mathbf{Q}}^{'}_1, \mathbf{A}_2, \widehat{\mathbf{S}}_2$,

$\mathbf{x}$: random vector,

$\mathbf{1_n}$: is a vector of n ones,

$\mathbf{\Sigma}$: covariance matrix

vec: vec-operator

tr: trace operator

$\dim(\bullet)$: dimension of a matrix

$\mathbf{G}^o$: is any matrix spanning the orthogonal complement to the space generated by the columns of $\mathbf{G}$,

$(\mathbf{G}^o)^{'}$: is the transpose of matrix $\mathbf{G}^o$,

$(\mathbf{G}^{'})^o$: is any matrix spanning the orthogonal complement to the space generated by the columns of $\mathbf{G}^{'}$

CRD: Completely Randomized Design

RCBD: Randomized Complete Block Design

RBD: Randomized Block Design

1.6 Scope, Limitations and Assumptions

In this section, the delimitation (scope), limitations and assumptions are presented.

1.6.1 Scope

This work covers the application of growth curve model to analyze the impact of Calcium foliar feed on the wilting rate of rose flowers over time. The data are obtained by doing split-split plot experiment. For more details about the data, see Lewis (2017). In this study, there are three main factors which are the covering material applied to the main plot, 2 varieties of rose flowers applied to the sub plots and the Calcium foliar feeds applied to the sub-sub plots. This study interests in one variety of rose flowers and foliar feed with 5 levels or treatment groups. The response variable is the wilting scores of rose flowers and the explanatory variable is the time. The growth curve model is also applied to estimate the trends or growth curves in all foliar feeds and to investigate if the Calcium foliar feed has significant effect of the wilting rate of rose flowers over time.

1.6.2 Limitations

This work does not consider the covering material factor. It does not consider application of linear regression models including linear mixed model for which investigates the relationship between the response and explanatory variables. This model is used to identify if there exists any interaction between the covering material and foliar feed. It is also used to identify which factor has significant effects on the wilting rate of rose flowers.

1.6.3 Assumptions

In practice, most data are not samples from normal distribution and they do not satisfy homoscedasticity assumption, that is why, the data transformation are needed. In such a case, the data are transformed using square root in this work because the data are considered as count data. In the GCM defined in (3.1), the columns of observation matrix $\mathbf{X}$ are assumed to be independent normally distributed p-vectors and columns of error matrix $\mathbf{E}$ are assumed to follow the normal distribution. In this model, the degrees of growth curves q are assumed to be the same in all foliar feeds, and the within-individual design matrix $\mathbf{A}$ and between-individual design matrix $\mathbf{C}$ are of full rank.

1.7 Outline of the thesis

This thesis is composed by five chapters as follows. Chapter one deals with introduction. Chapter two consists of the literature review on rose flowers, design of experiment and analysis, experimental design of this study. Chapter three covers the data and methods of the study. Chapter four deals with results and discussions and chapter five deals with concluding remarks.

Chapter 2

LITERATURE REVIEW

This chapter focuses on the background of the rose flower varieties, factors affecting the wilting process of rose flower varieties, the experimental Design and design of experiment and analysis.

2.1 Rose flowers

2.1.1 Rose flower varieties

Roses can be classified into 3 groups according to their growth characteristics. Those groups are **bush, climbing and shrub**.

Bush Roses

Bush roses are self supporting and bear flowers primarily at the top of the plant. Plant heights vary from a few meters to 1.83 meters [2]. According to the growth and flowering habits of the bush roses, the bush roses can be grouped as follows:

1. **Polyantha Rose**
   It is introduced in the late 19^{th} century. Plants are energetic, many branched. Polyantha Rose is usually low growing to 3 tall and it has small flowers but in large sprays, it gives mass of color. Five common varieties of Polyantha Rose are Margo Koster, The Fairy, China Doll, Cecile Brunner and Perle d' Or.

2. **Hybrid Tea Rose**
   It is the most popular class of rose grown today; sell more than all the other types combined. Hybrid Tea Rose was first introduced in 1867. Since its introduction, different varieties (thousands) of Hybrid Tea Rose was produced. The Hybrid Tea Rose has the following characteristics : long blooming season, $3-7$ tall plants that should be planted $2-3$ apart, large flowers $(3.5-5.5) meters$ produced one to a stem and accented by handsome foliage, etc. Different varieties of Hybrid Tea Rose are Mr. Lincoln, Peace, Touch of Class, Brandy, Double Delight.

3. **Floribunda Rose**
   It is the result of crosses between polyantha and hybrid tea and it is relatively short, plants are $2-3$ tall. Several floribundas planted 2 apart make a good hedge. $2.5-3.5$ flowers are borne in large clusters. It has the same bloom shape

as of hybrid tea. Clusters are abundant and provide mass of color. More color selections of floribunda are available than for polyanthas. Selections include Iceberg, Angel Face, Showbiz, Sun Flare, and Intrigue.

4. **Grandiflora Rose**
   It should be a cross between hybrid tea and floribunda, but it sometimes has extra energetic hybrid teas. Plants often grow $8-10$ tall, plant 6 apart and use as a background or barrier plant. Long blooming season with large clusters of $2.5-3.5$ flowers or single blooms if a hybrid tea. Varieties of Grandiflora Rose are Queen Elizabeth, Tournament of Roses, and Solitude are favorites [2]..

Climbing Roses

They are extremely vigorous plants. These plants have long branches which may range from 1.53 to 6.1 meters in size depending on the type of rose and how they are supported and maintained. Branches can be also trained to a trellis or fence or allowed to sprawl as a bank cover. There are classified into two main types:**natural climbers** and **climbing forms of hybrid teas**. Both types produce long branches that need support from walls, fences, etc. Some bloom repeatedly, others have one annual display. These plants are planted at least 6 apart and away from the others. The varieties of climbing roses are Lady Banks, Blaze, Dortmund, and Cl. Cecile Brunner. The varieties are the most popular in the world [2].

Shrub Roses

Shrub roses are characterized by non specific class of wild species, hybrids, and cultivars. According to these characteristics, shrub roses need a little maintenance in order to develop large and intense growth. Many have fine-textured foliage, making them suitable for use as hedges or screen plantings [2]. Figure 2.1 is an example of bush rose flowers which are found in Rwamagana District.

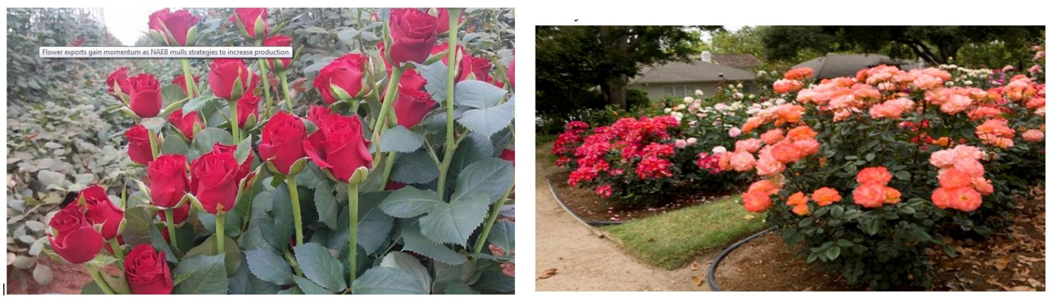

Figure 2.1: Different types of rose flowers (source:[3])

2.1.2 Factors affecting the quality and development of plants

There are several factors that affect the quality and development of plants include poly film covers, calcium foliar feed, environment factors like light, water, temperature, humidity, ventilation, etc. Those factors can also affect the wilting process of rose flowers varieties.

Poly film covers

Poly films are popularly known as polyethylene or agricultural plastic films. They are made from materials that are synthetic in origin. Poly film covers are mainly used in agriculture to provide visible separation between the outdoor and greenhouse environments in protected farming [7]. The aims of poly film covers are used to ensure the production of any plant at any place and throughout the year, control insect-pests and diseases incidences, enhance quality of produce, reduce crop duration, etc. As a result, the capability of the poly film to allow maximum possible light for better plant growth and development is of primary concern to most greenhouse growers. However, the intensity of solar radiation that penetrates the covering material to reach the plants is considerably affected by atmospheric water vapour, carbon dioxide concentrations, greenhouse structure and other environmental contaminants [8]. Light transmission is further affected by water condensate on the poly film cover and thickness of the cover. The effect of condensation is more on untreated poly film, the condensate occurs in small droplets which reduces light transmission from solar radiation through multiple reflections. Reduction in light transmission may occur further due to dirt deposition which accumulate during production and some light is also lost through reflection and absorption by greenhouse construction material. In addition, the degree of poly film degradation and shape of the structure being covered affect light transmission considerably [7].

Recently greenhouse production has embraced the use of cladded poly films which comprise of many additives. The technology involves addition of different additives during manufacture giving poly films of different colours and consequently spectra properties. This additive products enhance poly films to determine its durability, capacity to reduce heat loss, capacity to reduce droplet formation, transmission of particular wavelengths of light, capacity to reduce the amount of dust sticking to the film [9]. The following are the examples of different types of additives:

- UV ($290 - 400nm$) absorbers and stabilisers increase durability, reduce the potential damage to biological systems in the greenhouse and may control some plant pathogens,
- Infra-Red ($700 - 2500nm$) absorbers reduce long wave radiation and minimize heat loss,
- Long wave radiation ($2500 - 40000nm$) absorbers reduce the loss of heat radiated from materials and objects (including plants) inside the greenhouse,
- Light diffusers scatter light entering the greenhouse, reducing the risk of plants getting burnt and improving the amount of light available to the lower parts of the plant,
- Surfactants reduce the surface tension of water, dispersing condensation,
- Antistatic agents reduce the tendency of dirt to accumulate on plastic films.

Therefore, greenhouse growers must select appropriate poly film covers to avoid negative impact on the plants.

Effect of calcium fertilizer on plant growth and development

Calcium is an important plant nutrient as it forms calcium pectate a major constituent of the plant cell wall, helping to build a strong structure and ensuring cell stability, also important for cell elongation. Calcium is highly immobile, it is translocated through the transpiration stream. Factors that affect transpiration such as high relative humidity and low air temperature obviously affect calcium uptake and availability. Calcium contributes significantly to stem strength and minimize cases of cut flower breakages during post - harvest handling. Conventionally, plants take up nutrients from the soil through the roots. However, calcium is mostly unavailable to plants while it is available in adequate quantities in the soil solution due to poor distribution and difficulty in remobilization from old tissue to new tissues [7]. Advances in fertilizer application has led to the development of fertilizer supplements and nutrients that can now be applied directly to the leaves [10] with guaranteed availability of nutrients such as calcium that are hardly translocated in the plant system. The foliar nutrition is recognized as an important method of fertilization because the nutrients are immediately penetrate the leaf cuticle or stomata and enters the cells facilitating easy and rapid utilization of nutrients. One of the benefits of foliar fertilization is the increased uptake of the nutrients as opposed to soil application. The other interest in foliar fertilizers arose due to the multiple advantages of foliar application methods such as rapid and efficient response to the plant needs, less product needed and independence of soil conditions. It is also recognized that supplementary foliar fertilization during crop growth can improve the mineral status of plants and increase the crop yield [11]. Therefore, Calcium plays vital role in nearly all aspects of plant growth and development and reduce wilting process of rose flowers across the time.

2.2 The importance of rose flowers

There are so many functions of rose flowers. They are used as ornamental and commercial crops. The rose oil is used in manufacturing commercial perfumery, used as room refreshers. Rose flower also used as water rose which has different uses in medicines. It provides relief from soothes of mind, rose petals are antiseptic in nature, and used as an eye wash ingredient. It is enriched with vitamins such as vitamin A, C, D, E, and B. For skin beauty, rose water is used in face masks for fair skin which maintains skin pH and controls extra oil. If one cup of rose water is used for hair shining, along shining it produces healthy hairs and in jams, jellies and in soup rose water used. The rose is a sign of love and peace and used as a gift, also for welcome parties, wedding ceremonies, and many religious events [12].

2.3 Design of experiment and analysis

Definition 2.1 ***Designing*** *is a process or set of rules by which treatments are assigned to experimental units to verify or discard the hypothesis made by the experimenter.*

The purpose of designing of experiment is to increase the precision of the experiment, i.e, to get more and more information per observation. In order to increases the precision, the experimental error needs to be reduced.

Definition 2.2 *Experimental error is the unexplained random part of variation in any experiment. An estimate of experimental error can be obtained by replication.*

2.3.1 Procedure for designing an experiment

An outline of the stages that are involved in design and analysis of an experiment are provided in [13] as follows:

Recognition of the statement of the problem

It is necessary to develop all ideas about the objectives of the experiment. A clear statement of the problem often contributes much in exploring the phenomena and the final solution to the problem.

Choice of factors and levels

This involves a process of selecting independent variables or factors to be investigated in the experiment. Levels may be chosen specifically at random from the set of all possible factor levels.

Selection of response variable

In choosing a response variable, the experimenter ensures that the response to be measured really provides information of the underlying problem.

Choice of experimental design

The experimenter should determine the difference between each design and the magnitudes of risks that are associated with each design as well as the benefits they have. A provisional mathematical model of the experiment must also be proposed to the data of interest before beginning the analysis of the data.

Performing the experiment

This is the actual data collection process in which the experimenter carefully monitors the progress of the experiment to ensure that it is proceeding according to the design plan. Particular attention must be paid to the principles of the experiment, measurements, accuracy and making as uniform an experimental environment as possible.

Data analysis

In general statistical methods cannot prove that a factor has particular effect but only provide guidelines as to the reliability and validity of results. When properly used statistical methods allow measures that will result in a sound conclusion.

2.3.2 Basic principles of experimental designs

The techniques used to reduce experimental error or to increase the precession of experiment form the basic principles of experimental designs. This section presents the three principles of the experimental design as follows.

Randomization

Randomization is process of allocating treatments to various experimental units randomly so that each treatment gets an equal opportunity to show its worth. The purpose of randomization is to assure that sources of variation due to extraneous factors operate randomly so that the average effect on any group of units is zero. Thus randomization technique ensures unbiasness of the estimate of experimental error. The randomization procedure for the split-split plot arrangement consists of three parts [14]:

- Main plot treatments are randomly assigned to main plots based on the design used.
- Split plot treatments are randomly assigned to the subplots.
- Split-split plot treatments are randomly assigned to the sub-subplots.

The randomization process is independent of each portion. In split-split plot designs randomization is restricted twice. The experimental design used to randomize the whole plots will not affect randomization of the sub and sub-subplots.

Replication

Repetition of treatment to different experimental units is known as **replication**. The benefits of replication in experimental design include:

- It allows the experimenter to obtain an estimate of the experimental error which is regarded as the basic unit of measurement for determining whether the observed are statistically different.
- Replication of treatment reduces experimental error.
- If the sample mean is used to estimate the effect of a factor in the experiment then replication permits the experimenter to obtain a more precise estimate of the effect.

In general replication is best for the experiment that it allows for the accurate estimation of the controllable factor level means. More precisely, it improves the sensitivity test for comparing factor level means [15].

Local control or Blocking

Grouping of homogeneous experimental units is known as **local control**. The local control helps in reduction of experimental error. In agricultural field experiments, the neighboring plots expected to have homogeneous environmental conditions such as soil fertility, depth of soil for examples and hence the neighboring plots could be grouped into blocks. In animal experiments, animals having same age, litter, sex, lactation for examples could be grouped. This grouping of homogeneous experimental units will reduce the experimental error as the differences between blocks can be removed from the experimental error in the analysis of variance table. The commonly used techniques for controlling experimental error in agricultural research are:

- Selection of experimental material,
- Proper plot technique (shape and size of plots),
- Data analysis (competition effect).

Those techniques can be founded in [16].

2.3.3 Split plot Design

Split plot design (SPD) is a factorial design with at least two factors where the experimental unit with respect to factors differ in size or observation points. Split-plot experiments are usual practical not exceptions in the field of agriculture. In simple terms, a split-plot experiment is a blocked experiment, where the blocks themselves serve as experimental units for a subset of the factors. This shows that there are two levels of experimental units in the split-plot experiments. The blocks are referred to as whole plots, while the experimental units within blocks are called split plots, split units or subplots. Corresponding to the two levels of experimental units are two levels of randomization. One randomization is conducted to determine the assignment of block-level treatments to whole plots. Then, as always in a blocked experiment, a randomization of treatments to split-plot experimental units occurs within each block or whole plot see Figure 2.2 for further understanding where A and B are whole and split plots respectively [17].

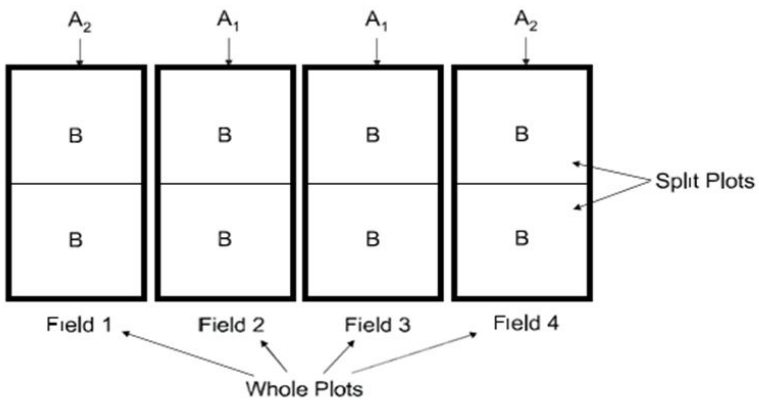

Figure 2.2: Split plot Design (source: [18])

The split-plot design is closely related to the CRD and RCBD. A CRD design is where the study subjects are assigned to treatments in a random manner and there is no restriction on the assignment of treatments to the plots, that is, with the CRD, every plot is equally likely to be assigned to any treatment. In constructing RCBD or RBD, the plots are grouped into blocks. Normally, the number of plots in each block is equal to the number of treatments, while the number of blocks is equal to the number of replications per treatment. That is, in the usual RBD each treatment occurs once, and only once, in each block. Once the blocks have been formed, the treatments are assigned at random to the plots within the blocks [18].

2.3.4 Split-split plot Design

Split-Split Plot design (SSPD) is an extension of split plot to include a third factor. In agriculture, it is ideal to include a third factor in order to have adequate evidence of the prevailing situation. In practice there is no restriction on the extensions that can be done so as to incorporate as many factors as we can in order to gain sufficient information on how different factors interact. However, the analysis becomes cumbersome as one incorporates more factors. The structure of a split-split plot is described by:

- Different sizes of plots; which are the biggest plot for the whole plot factor, the middle sized plot for the split plot factor, and the smallest for the split-split plot factor.
- The main factor receive the least precision, and the split-split plot factor receive the most precision.

For example, split-split plot arrangement with two levels of the whole plot factor, A, two levels of the subplot factor, B, and six levels of the sub-subplot factor, C. The first replicate is shown in Figure 2.3, where α_i, β_j and τ_k represent the factor levels

	Subplot	Sub-subplot					
α_1 whole plot	$\alpha_1\beta_1$	$\alpha_1\beta_1\tau_1$	$\alpha_1\beta_1\tau_2$	$\alpha_1\beta_1\tau_3$	$\alpha_1\beta_1\tau_4$	$\alpha_1\beta_1\tau_5$	$\alpha_1\beta_1\tau_6$
	$\alpha_1\beta_2$	$\alpha_1\beta_2\tau_1$	$\alpha_1\beta_2\tau_2$	$\alpha_1\beta_2\tau_3$	$\alpha_1\beta_2\tau_4$	$\alpha_1\beta_2\tau_5$	$\alpha_1\beta_2\tau_6$

Figure 2.3: The first replicate for split-split plot arrangement (source: [19])

i,j and k of whole plot, subplot and sub-subplot, respectively, for $i = 1$; $j = 1, 2$ and $k = 1, 2, \ldots, 6$.

2.4 The experimental design of the study

In this thesis, there is an experimental design of the form of a split-split plot design. In this design, there were covering material with 3 levels (polythene cover 1, 2 and 3), 2 varieties of plants (rose flower variety 1, and 2) and 5 types of foliar feeds, each with different concentration levels of calcium. The experiment was set up in 3 plots (main plots) contained in block factor (3 levels). Each of the main plots was split into 2 sub plots (split-plots) and each of these sub plots was split into 5 sub-sub plots (split-split-plots). Each type of the covering material was systematically assigned in each of the main plots, each level of the plant variety was systematically assigned to the sub plots and each of the foliar feeds was systematically assigned to the sub-sub plots. Therefore, the foliar feeds were contained in variety and the varieties were contained in the covering materials. The entire experiment was conducted in 4 different time periods (known as day 0, day 3, day 6 and day 9) with the same runs and the results recorded. Below are the experimental conditions used in the wilting experiments:

1. Rose flower varieties (V):

- Rose variety 1 (V_1)
- Rose variety 2 (V_2)

2. Covering materials (G):
    - Polythene cover 1 (G_1)
    - Polythene cover 2 (G_2)
    - Polythene cover 3 (G_3)
3. Foliar feeds (T):
    - Nutrient feeding calcium level 1 (T_1)
    - Nutrient feeding calcium level 2 (T_2)
    - Nutrient feeding calcium level 3 (T_3)
    - Nutrient feeding calcium level 4 (T_4)
    - Nutrient feeding calcium level 5 (T_5)

From this design, we observe that the three levels of the covering material (G) were applied in the main plot. Then, the two levels of the rose flower varieties (V) were assigned in each of the plots where each of the levels of the covering material was applied. Lastly, the five levels of the foliar feeds (T) were applied in each of the sub plots where each of the rose varieties was applied. This means that each of the 3 plots in the main plot was split into 2 sub plots and each of these sub plots was split into 5 sub-sub plots. Therefore, the variance components of the error are determined by the plot, sub plot which is the pair of columns in the experimental layout and the sub-sub plot (random error) which are the columns within each pair of columns. Such an experiment is called a split-split plot experiment and the design is called a split-split plot design [20].

Table 2.1: Treatment Design (source:[5]).

$G_1V_1T_1$	$G_1V_2T_1$	$G_2V_1T_1$	$G_2V_2T_1$	$G_3V_1T_1$	$G_3V_2T_1$
$G_1V_1T_2$	$G_1V_2T_2$	$G_2V_1T_2$	$G_2V_2T_2$	$G_3V_1T_2$	$G_3V_2T_2$
$G_1V_1T_3$	$G_1V_2T_3$	$G_2V_1T_3$	$G_2V_2T_3$	$G_3V_1T_3$	$G_3V_2T_3$
$G_1V_1T_4$	$G_1V_2T_4$	$G_2V_1T_4$	$G_2V_2T_4$	$G_3V_1T_4$	$G_3V_2T_4$
$G_1V_1T_5$	$G_1V_2T_5$	$G_2V_1T_5$	$G_2V_2T_5$	$G_3V_1T_5$	$G_3V_2T_5$

Table 2.1 shows that the levels of the flower variety do not change in each of the levels of the covering material. This means that the covering material and variety are crossed factors. In addition, each of the levels of the foliar feeds do not change in each of the levels of the variety implying that the foliar feed and variety are also crossed. The previous researcher is used linear mixed-effects models to analysis the effect of covering material and foliar feeds on the wilting rate of rose flowers varieties and the interaction between those two factors. He found that the wilting process is affected by the foliar feeds but not covering material. He also found the interaction between the cover and feed is not significant in the model. The interaction in this context examines if the effect of changing from one type of covering material to another is different in different foliar feeds. This experiment

was done by International Centre for Tropical Agriculture Rwanda and it can be found in [5]. But, he did not consider the wilting process of rose flowers across the time because this method of analysis was not able to take into account the patterns of change of wilting rate over time. That is why, this work will apply growth curve model to analyse the effects of calcium foliar feed on the wilting rate of rose flowers over time.

Chapter 3

DATA AND METHODS

3.1 Data description

The data under consideration is a secondary data obtained from International Centre for Tropical Agriculture (CIAT) Rwanda. I didn't present the data in Annex because I am not allowed to publish them, for further explanation and clarification about the data, you can visit CIAT Rwanda. The data consist of 5 levels of Calcium foliar feed each with different concentration. Note that each level is considered as treatment group and each treatment group consists of 9 rose flowers of the same variety and the wilting scores for every rose flower is repeatedly measured 4 different time points (i.e days 0,3,6 and 9). This shows that the data are balanced. The response variable (variable of interest) was the wilting scores of rose flowers and the wilting was quantified by assigning a score ranging between 1 (least wilted) and 5 (most wilted). In each of these days, the leaves were observed and assigned a score which corresponded to their level of wilting. The independent variable is time. In this work, the average of the wilting scores of rose flowers at each time is referred to the wilting rate and concentration of Calcium foliar feed is referred to foliar feed. In this work, the vector and matrix Matlab commands are **eye, ones, inv, pinv, det**; the plotting commands are **plot, Xlabel, Ylabel, hold on and legend** and the statistical Matlab function are **min** and **std** . These commands are used to carry the calculations in order to perform descriptive and inferential analysis of the interest data.

3.2 Model formulation

In this section, model description, Maximum Likelihood Estimators and general linear test of hypothesis will be presented.

3.2.1 Growth curve model

The Growth Curve Model (GCM) is a Generalized Multivariate Analysis of Variance (GMANOVA) model. This model is used for analyzing longitudinal data, growth curves or trends and other data with repeated measurements. It is particularly useful when we have short to moderate time series where one cannot apply standard time series approaches [21]. The mean structure for GCM is bilinear, this means that, the two design matrices are involved, in contrary for the ordinary Multivariate

Analysis of Variance (MANOVA) model where the mean structure is linear, that is, one design matrix is involved ([21], [22], [23], [24], [25]). The model was first introduced by Potthoff and Roy [26] in 1964. Since its introduction, the model has been extensively explored by many authors including [22], [27], [28], [29], [30], [31], [32], for examples. The model is particularly useful when we have short to moderate time series where one cannot apply standard time series [33]. The repeated measurements over time lead to a within-individual model. However, it is also essential to consider a between-individual model as the repeated measurements are recorded on several individuals, often distributed across more than one group. With the inclusion of a between individual design matrix, the model becomes bilinear [22]. GCMs have extensive applications in the field of economics, social and natural sciences, medical research, epidemiology, psychology, pharmaceutical studies and agriculture ([21], [22], [33], [34], [35], [36]). It is to be noted that the GCM, like any other model involves several assumptions and limitations. One of the assumptions is that the GCM assumes the same degree of polynomial in time across all the treatment groups. That is, it assumes the expected response in each of the groups to be a q^{th} order polynomial of the continuous predictor variable, say, **time**. However, in practice, this assumption might often be violated when responses from different groups have different shapes, and hence different degrees of polynomials. Another model is, therefore, required to put up the need for this difference across group means. In this thesis, we will assume that the degrees of the growth curves are the same in all foliar feeds. Moreover, it can be useful whenever there is a focus on the analysis of change over time.

3.2.2 Model Description

Definition 3.1 *Let* $\mathbf{X} : p \times n$ *and* $\mathbf{B} : q \times k$ *be the observation and parameters matrices, respectively, and let* $\mathbf{A} : p \times q$ *and* $\mathbf{C} : k \times n$ *be the within and between-individual design matrices, respectively. Suppose that* $r(C) + p \leq n$ *and* $q \leq p$. *The growth curve model is defined by*

$$\mathbf{X} = \mathbf{ABC} + \mathbf{E}, \tag{3.1}$$

where $\mathbf{E} : p \times n$ *is the error matrix and its columns are assumed to be independent* p *variates normally distributed with zero mean and an unknown positive definite covariance matrix* $\mathbf{\Sigma}$, *i.e,*

$$\mathbf{E} \sim N_{p,n}(\mathbf{0}, \mathbf{\Sigma}, \mathbf{I}_n).$$

It is important to note that the matrix of unknown parameters $\mathbf{B}$ consisting of the coefficients of the polynomials described in (3.7) whereas $\mathbf{A}$ and $\mathbf{C}$ are known design matrices. Moreover, the between individual design matrix $\mathbf{C}$ is precisely the same design matrix as used in the theory of univariate and multivariate linear models which includes univariate analysis of variance and regression models. If $\mathbf{A} = \mathbf{I}$, the growth curve model reduces to the usual MANOVA model treated in most texts on multivariate analysis [22]. Observe that the different columns of $\mathbf{X}$ are assumed to be independent normally distributed with mean $\mathbf{ABC}$ i.e ($E(\mathbf{X}) = \mathbf{ABC}$) and an unknown positive definite covariance matrix $\mathbf{\Sigma}(p \times p)$, that is, $\mathbf{X} \sim N_{p,n}(\mathbf{ABC}, \Sigma, \mathbf{I}_n)$. Each matrix involved in the model (3.1) can be written in the following form

i. The observation matrix $\mathbf{X}$ is

$$\mathbf{X} = \begin{pmatrix} x_{11} & x_{12} & \dots & x_{1n} \\ x_{21} & x_{22} & \dots & x_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ x_{p1} & x_{p2} & \dots & x_{pn} \end{pmatrix} = \begin{pmatrix} \mathbf{x}_1 & \mathbf{x}_2 & \dots & \mathbf{x}_n \end{pmatrix} \tag{3.2}$$

with $\mathbf{x}_i = (x_{1i}, x_{2i}, \dots, x_{pi})', i = 1, 2, \dots, n.$

ii. The within-individuals design matrix $\mathbf{A}$ is defined as

$$\mathbf{A} = \begin{pmatrix} 1 & t_1 & \dots & t_1^{q-1} \\ 1 & t_2 & \dots & t_2^{q-1} \\ \vdots & \vdots & \ddots & \vdots \\ 1 & t_p & \dots & t_p^{q-1} \end{pmatrix}. \tag{3.3}$$

iii. The matrix of unknown parameters $\mathbf{B}$ is

$$\mathbf{B} = \begin{pmatrix} \beta_{11} & \beta_{12} & \dots & \beta_{1k} \\ \beta_{21} & \beta_{22} & \dots & \beta_{2k} \\ \vdots & \vdots & \ddots & \vdots \\ \beta_{q1} & \beta_{q2} & \dots & \beta_{qk} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\beta}_1 & \boldsymbol{\beta}_2 & \dots & \boldsymbol{\beta}_k \end{pmatrix}. \tag{3.4}$$

iv. The between-individuals design matrix $\mathbf{C}$ can be written as

$$\mathbf{C} = \begin{pmatrix} 1 & \dots & 1 & 0 & \dots & 0 \dots & 0 & 0 & \dots & 0 \\ 0 & \dots & 0 & 1 & \dots & 1 \dots & 0 & 0 & \dots & 0 \\ \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots & \dots \\ 0 & \dots & 0 & 0 & \dots & 0 \dots & 1 & 1 & \dots & 1 \end{pmatrix}$$
$$= \left(\mathbf{1}'_{n_1} \otimes (1 : 0 : \dots : 0)' : \mathbf{1}'_{n_2} \otimes (0 : 1 : 0 : \dots : 0)' : \dots : \mathbf{1}'_{n_k} \otimes (0 : 0 : \dots : 1)'\right).$$

v. The error matrix $\mathbf{E}$ is defined by

$$\mathbf{E} = \begin{pmatrix} \epsilon_{11} & \epsilon_{12} & \dots & \epsilon_{1n} \\ \epsilon_{21} & \epsilon_{22} & \dots & \epsilon_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ \epsilon_{p1} & \epsilon_{p2} & \dots & \epsilon_{pn} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\epsilon}_1 & \boldsymbol{\epsilon}_2 & \dots & \boldsymbol{\epsilon}_n \end{pmatrix}. \tag{3.5}$$

Now, let us formulate the growth curve model of this study. Let x_{ik} denote the wilting scores of rose flowers measured on subject i at time t_j with $j = 1, 2, 3, 4$. The summary statistics are calculated by using the following formulas: First, the sample mean at the j^{th} time point is

$$\bar{x}_{\cdot j} = \frac{1}{n} \sum_{i=1}^{n} x_{ij}, \tag{3.6}$$

where the . subscript indicates averaging over the first index i. The sample mean can be calculated for each time point j and the sample mean $\bar{\mathbf{x}}$ is given by

$$\bar{\mathbf{x}} = \frac{1}{n}\sum_{i=1}^{n}\mathbf{x}_i = (\bar{x}_{.1}, \bar{x}_{.2}, \ldots \bar{x}_{.p})^{'}.$$

Moreover, the wilting of rose flowers have been scored at 4 different time points (i.e day 0,3,6 and 9). This shows that $p = 4$. The wilting scores of rose flowers in all foliar feeds are measured at the same p points in time. This is a necessary condition for applying the Growth Curve model. The growth curve associated with the k^{th} group is assumed to be a polynomial in time t of degree $q-1$, so that the expected value for k^{th} group at time t is

$$\mu_k = \beta_{1k} + \beta_{2k}t + \ldots + \beta_{qk}t^{q-1}, \tag{3.7}$$

where β_{ik} are the unknown parameters with $i = 1, 2, \ldots, q$ and $k = 1, 2, \ldots, 5$. Furthermore, data form a random matrix $\mathbf{X} = (\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_{45})$ in which nine first columns correspond to measurements on foliar feed 1, nine second columns correspond to measurements on foliar feed 2, nine third columns correspond to measurements on foliar feed 3, nine fourth columns correspond to measurements on foliar feed 4 and nine fifth columns correspond to measurements on foliar feed 5. The matrix of unknown parameters $\mathbf{B}$ will be defined as in (4.3) with $k = 5$ and the within-individuals design matrix $\mathbf{A}$ will be defined as in (3.3) with $p = 4$. The between-individuals design matrix $\mathbf{C}$ has $5 \times n$ dimension and it is defined as $\mathbf{C} = (\mathbf{1}^{'}_{n_1} \otimes (1:0:\ldots:0)^{'} : \mathbf{1}^{'}_{n_2} \otimes (0:1:0:\ldots:0)^{'} : \ldots : \mathbf{1}^{'}_{n_5} \otimes (0:0:\ldots:1)^{'})$

3.2.3 Method of estimation of parameters

In this part, the Maximum Likelihood Estimators (MLE) for the mean parameter $\mathbf{B}$ and $\mathbf{\Sigma}$ will be derived. This part is mainly interested in the mean structure and we suppose that there is no information about any structure in $\mathbf{\Sigma}$. Consider a sample $\mathbf{X} = (\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_n)$ for which it follows $N_{p,n}(\mathbf{ABC}, \mathbf{\Sigma}, \mathbf{I}_n)$, the density function of $\mathbf{X}$ is defined by

$$f(\mathbf{X}, \mathbf{B}, \mathbf{\Sigma}) = (2\pi)^{-\frac{1}{2}np}|\mathbf{\Sigma}|^{-\frac{n}{2}} \exp\left\{-\frac{1}{2}\,\mathrm{tr}[\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})^{'}]\right\}. \tag{3.8}$$

Its likelihood function denoted $L(\mathbf{B}; \mathbf{\Sigma})$ is defined by

$$L(\mathbf{B}, \mathbf{\Sigma}) = (2\pi)^{-\frac{1}{2}np}|\mathbf{\Sigma}|^{-\frac{n}{2}} \exp\left\{-\frac{1}{2}\,\mathrm{tr}[\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})^{'}]\right\}. \tag{3.9}$$

The MLE of $\mathbf{B}$ and covariance $\mathbf{\Sigma}$ matrices are obtained by maximizing the likelihood function. Instead of maximizing this product which can be quite tedious, it will be equivalent to maximize the log-likelihood function ($\ln L$) [37] since the logarithm is always a monotonically increasing function, that is, one need to maximize

$$\ln L(\mathbf{B}; \mathbf{\Sigma}) = -\frac{np}{2}\ln(2\pi) + \frac{n}{2}\ln|\mathbf{\Sigma}^{-1}| - \frac{1}{2}\,\mathrm{tr}[\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})^{'}]. \tag{3.10}$$

Let us find the corresponding likelihood equations with respect to $\mathbf{B}$ and $\mathbf{\Sigma}$ by differentiating the function (3.10) with respect to $\mathbf{B}$, $\mathbf{\Sigma}$ and equating to zero. First, differentiate function (3.10) with respect to $\mathbf{B}$. That is

$$\frac{\partial}{\partial \mathbf{B}} \ln L(\mathbf{B}, \mathbf{\Sigma}) = -\frac{1}{2} \frac{\partial}{\partial \mathbf{B}} \operatorname{tr}[\Sigma^{-1}(\mathbf{X} - \mathbf{ABC})(\mathbf{X} - \mathbf{ABC})^{'}]. \tag{3.11}$$

Let $P = \mathbf{\Sigma}^{-1}(\mathbf{X} - \mathbf{ABC})(\mathbf{X} - \mathbf{ABC})^{'}$, the equation (3.11) transforms to

$$\frac{\partial}{\partial \mathbf{B}} \ln L(\mathbf{B}, \mathbf{\Sigma}) = -\frac{1}{2} \frac{\partial \operatorname{tr} \mathbf{P}}{\partial \mathbf{B}} = -\frac{1}{2} \frac{\partial P}{\partial \mathbf{B}} \operatorname{vec} \mathbf{I},$$

since $\frac{\partial \operatorname{tr} \mathbf{Y}}{\partial \mathbf{X}} = \frac{\partial \mathbf{Y}}{\partial \mathbf{X}} \operatorname{vec} \mathbf{I}$, then the properties of matrix derivatives give

$$\begin{aligned}
\frac{\partial \mathbf{P}}{\partial \mathbf{B}} &= \frac{\partial}{\partial \mathbf{B}} [\mathbf{\Sigma}^{-1}(\mathbf{X} - \mathbf{ABC})][(\mathbf{X} - \mathbf{ABC})^{'} \otimes \mathbf{I}] \\
&+ \frac{\partial}{\partial \mathbf{B}} [(\mathbf{X} - \mathbf{ABC})^{'}][\mathbf{I} \otimes (\mathbf{\Sigma}^{-1}(\mathbf{X} - \mathbf{ABC}))'] \\
&= \mathbf{C}\,(\mathbf{X} - \mathbf{ABC})' \otimes \left(\mathbf{\Sigma}^{-1}\mathbf{A}\right)' \\
&- K_{q,k}\,(\mathbf{A} \otimes \mathbf{C})\,[\mathbf{I} \otimes (\mathbf{\Sigma}^{-1}(\mathbf{X} - \mathbf{ABC}))'] \\
&= \mathbf{C}\,(\mathbf{X} - \mathbf{ABC})' \otimes \left(\mathbf{\Sigma}^{-1}\mathbf{A}\right)' - K_{q,k} \left\{ \mathbf{A} \otimes [\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}']' \right\},
\end{aligned}$$

where $K_{q,k}$ is a commutation matrix,

$$\frac{\partial \mathbf{P}}{\partial \mathbf{B}} \operatorname{vec}\ \mathbf{I} = \left\{ \left[(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}')' \otimes \left(\mathbf{\Sigma}^{-1}\mathbf{A}\right)' \right] - K_{q,k} \left[\mathbf{A} \otimes \left(\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}'\right]' \right\} \operatorname{vec}\ \mathbf{I}. \tag{3.12}$$

Since $\operatorname{vec}(\mathbf{ABC}) = (\mathbf{C}' \otimes \mathbf{A}) \operatorname{vec}\ \mathbf{B}$ and $\frac{\partial \mathbf{P}}{\partial \mathbf{B}} \operatorname{vec} \mathbf{I} = \mathbf{0}$, then the equation (3.12) becomes

$$\begin{aligned}
-\operatorname{vec}\ \left(\mathbf{A}\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}'\right) - \operatorname{vec}\ \left(\mathbf{A}\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}'\right) = \mathbf{0}, \\
\operatorname{vec}\ \left[\mathbf{A}'\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}'\right] = \operatorname{vec}\ (\mathbf{0}).
\end{aligned}$$

Therefore, after differentiating function (3.10) with respect to $\mathbf{B}$, we get

$$\mathbf{A}'\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,\mathbf{C}' = \mathbf{0}. \tag{3.13}$$

Next, differentiate equation (3.10) with respect to $\mathbf{\Sigma}$

$$\begin{aligned}
\frac{\partial \ln L(\mathbf{B}, \mathbf{\Sigma})}{\partial \mathbf{\Sigma}} &= -\frac{n}{2} \frac{\partial |\mathbf{\Sigma}|}{\partial \mathbf{\Sigma}} \frac{1}{|\mathbf{\Sigma}|} - \frac{1}{2} \frac{\partial}{\partial \mathbf{\Sigma}} tr\ [\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,(\mathbf{X} - \mathbf{ABC})'] \\
&= -\frac{n}{2} \operatorname{vec}\left(\mathbf{\Sigma}^{-1}\right) - \frac{1}{2} \frac{\partial P}{\partial \mathbf{\Sigma}} \operatorname{vec}\ \mathbf{I} \\
&= -\frac{n}{2} \operatorname{vec}\left(\mathbf{\Sigma}^{-1}\right) + \frac{1}{2} \left[\mathbf{\Sigma}^{-1} \otimes \left(\mathbf{\Sigma}'\right)^{-1} \right] \left[(\mathbf{X} - \mathbf{ABC})\,(\mathbf{X} - \mathbf{ABC})' \otimes \mathbf{I} \right] \operatorname{vec}\ \mathbf{I} \\
&= -\frac{n}{2} \operatorname{vec}\left(\mathbf{\Sigma}^{-1}\right) + \frac{1}{2} \left[\mathbf{\Sigma}^{-1}\,(\mathbf{X} - \mathbf{ABC})\,(\mathbf{X} - \mathbf{ABC})' \otimes \left(\mathbf{\Sigma}^{-1}\right) \right] \operatorname{vec}\ \mathbf{I},
\end{aligned}$$

since $\mathbf{\Sigma}$ is symmetric,

$$\begin{aligned}\frac{\partial \ln L(\mathbf{B},\Sigma)}{\partial \mathbf{\Sigma}} &= -\tfrac{n}{2}\operatorname{vec}\mathbf{\Sigma}^{-1} + \tfrac{1}{2}\left\{[(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})'\left(\mathbf{\Sigma}^{-1}\right)]' \otimes \mathbf{\Sigma}^{-1}\right\}\operatorname{vec}\ \mathbf{I} \\ &= -\tfrac{n}{2}\operatorname{vec}\left(\mathbf{\Sigma}^{-1}\right) + \tfrac{1}{2}\operatorname{vec}\left(\mathbf{\Sigma}^{-1}\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})'\right).\end{aligned}$$

By taking $\dfrac{\partial \ln L(B,\mathbf{\Sigma})}{\partial \mathbf{\Sigma}} = 0$, we get

$$\begin{aligned}-\frac{n}{2}\operatorname{vec}\left(\mathbf{\Sigma}^{-1}\right) + \frac{1}{2}\operatorname{vec}\left(\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})'\mathbf{\Sigma}^{-1}\right) &= \mathbf{0}, \\ n\mathbf{\Sigma}^{-1} &= \mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})'\mathbf{\Sigma}^{-1}.\end{aligned}$$

Multiply twice $\mathbf{\Sigma}$ on each side , we get

$$n\mathbf{\Sigma} = (\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})^{'}.$$

Hence, the likelihood equations are the following:

$$\mathbf{A}'\mathbf{\Sigma}^{-1}(\mathbf{X}-\mathbf{ABC})\mathbf{C}' = \mathbf{0}, \tag{3.14}$$

and

$$n\mathbf{\Sigma} = (\mathbf{X}-\mathbf{ABC})(\mathbf{X}-\mathbf{ABC})'. \tag{3.15}$$

Observe that $\mathbf{\Sigma}$ has been estimated as a function of the mean.

Solutions of the likelihood equations

The equation (3.15) can be divided into two parts in the book [22] as follows

$$n\mathbf{\Sigma} = \mathbf{S} + \mathbf{VV}^{'}, \tag{3.16}$$

where the sum of squares matrix

$$\mathbf{S} = \mathbf{X}(\mathbf{I} - \mathbf{C}^{'}(\mathbf{CC}^{'})^{-}\mathbf{C})\mathbf{X}^{'}), \tag{3.17}$$

and

$$\mathbf{V} = \mathbf{XC}^{'}(\mathbf{CC}^{'})^{-}\mathbf{C} - \mathbf{ABC}, \tag{3.18}$$

where $(\mathbf{CC}^{'})^{-}$ stands for the generalized inverse of matrix $(\mathbf{CC}^{'})$. They note that $\mathbf{S}$ did not depend on the parameter $\mathbf{B}$. And [23], in their paper, they defined the two jointly sufficient statistics for estimating parameters in the GCM and the distributions of them. Those statistics are the mean $\mathbf{XC}^{'}(\mathbf{CC}^{'})^{-1}\mathbf{C}$ and $\mathbf{S}$ Therefore, to find the value of matrix $\mathbf{B}$ in the equation (3.15), Theorem 3.1 is applied.

Theorem 3.1 *A representation of the general solution of the consistent equation in* $\mathbf{X}$*:*

$$\mathbf{AXB} = \mathbf{C} \tag{3.19}$$

is given by any of the following three formulas:

$$\mathbf{X} = \mathbf{X}_0 + (\mathbf{A}^{'})^{o}\mathbf{Z}_1\mathbf{B}^{'} + \mathbf{A}^{'}\mathbf{Z}_2\mathbf{B}^{o'} + (\mathbf{A}^{'})^{o}\mathbf{Z}_3\mathbf{B}^{o'}$$
$$\mathbf{X} = \mathbf{X}_0 + (\mathbf{A}^{'})^{o}\mathbf{Z}_1 + \mathbf{A}^{'}\mathbf{Z}_2\mathbf{B}^{o'}$$
$$\mathbf{X} = \mathbf{X}_0 + \mathbf{Z}_1\mathbf{B}^{o'} + (\mathbf{A}^{'})^{o}\mathbf{Z}_2\mathbf{B}^{'}$$

where $\mathbf{X}_0 = \mathbf{A}^{-}\mathbf{C}\mathbf{B}^{-}$ *is a particular solution and* $\mathbf{Z}_i; i = 1; 2; 3,$ *stand for arbitrary matrices of proper sizes.*

The proof of this theorem can be found in [22]. We observe that the equation in (3.15) is linear in $\mathbf{B}$. After some calculations and from Theorem 3.1, it follows that the general solution of (3.15) is given by

$$\widehat{\mathbf{B}} = (\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-}\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{X}\mathbf{C}^{'}(\mathbf{C}\mathbf{C}^{'})^{-} + (\mathbf{A}^{'})^{o}\mathbf{Z}_1 + \mathbf{A}^{'}\mathbf{Z}_2\mathbf{C}^{o'}, \tag{3.20}$$

where $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are arbitrary matrices. If $\mathbf{A}$ and $\mathbf{C}$ are of full rank, i.e $r(\mathbf{A}) = q$ and $r(\mathbf{C}) = k$, a unique maximum likelihood estimator of $\mathbf{B}$ exists and is given by

$$\widehat{\mathbf{B}} = (\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-1}\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{X}\mathbf{C}^{'}(\mathbf{C}\mathbf{C}^{'})^{-1}. \tag{3.21}$$

Furthermore, the maximum likelihood estimator of $\mathbf{\Sigma}$ is given by

$$\begin{aligned} n\widehat{\mathbf{\Sigma}} &= (\mathbf{X} - \mathbf{A}\widehat{\mathbf{B}}\mathbf{C})(\mathbf{X} - \mathbf{A}\widehat{\mathbf{B}}\mathbf{C})^{'} = \mathbf{S} + \widehat{\mathbf{V}}\widehat{\mathbf{V}^{'}} \\ &= \mathbf{S} + \left[\mathbf{I} - \mathbf{A}(\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-}\mathbf{A}^{'}\mathbf{S}^{-1}\right]\mathbf{X}\mathbf{C}^{'}(\mathbf{C}\mathbf{C}^{'})^{-}\mathbf{C}\mathbf{X}^{'} \\ &\left[\mathbf{I} - \mathbf{S}^{-1}\mathbf{A}(\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-}\mathbf{A}^{'}\right]. \end{aligned}$$

Therefore, the maximum likelihood estimator of $\mathbf{\Sigma}$ is

$$n\widehat{\mathbf{\Sigma}} = \mathbf{S} + (\mathbf{I} - \mathbf{P}_{\mathbf{A},\mathbf{S}^{-1}})\mathbf{X}\mathbf{P}_{\mathbf{C}^{'}}\mathbf{X}^{'}(\mathbf{I} - \mathbf{P}_{\mathbf{A},\mathbf{S}^{-1}})^{'}, \tag{3.22}$$

where $\widehat{\mathbf{V}}$ is the matrix obtained by replacing $\mathbf{B}$ with $\widehat{\mathbf{B}}$, i.e,

$$\widehat{\mathbf{V}} = \mathbf{X}\mathbf{C}^{'}(\mathbf{C}\mathbf{C}^{'})^{-}\mathbf{C} - \mathbf{A}\widehat{\mathbf{B}}\mathbf{C}. \tag{3.23}$$

The estimated mean ($\mathbf{A}\widehat{\mathbf{B}}\mathbf{C}$) can be obtained by multiplying matrix $\mathbf{A}$ on the left hand side and matrix $\mathbf{C}$ on the right hand side of both sides of equation (3.21), respectively, we get

$$\mathbf{A}\widehat{\mathbf{B}}\mathbf{C} = \mathbf{A}(\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-1}\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{X}\mathbf{C}^{'}(\mathbf{C}\mathbf{C}^{'})^{-1}\mathbf{C} = \mathbf{P}_{\mathbf{A},\mathbf{S}^{-1}}\mathbf{X}\mathbf{P}_{\mathbf{C}^{'}}, \tag{3.24}$$

where $\mathbf{P}_{\mathbf{A},\mathbf{S}^{-1}} = \mathbf{A}(\mathbf{A}^{'}\mathbf{S}^{-1}\mathbf{A})^{-1}\mathbf{A}^{'}\mathbf{S}^{-1}$.
The estimated mean is always unique i.e. the expression does not depend on the choices of generalized inverses, therefore $\widehat{\mathbf{\Sigma}}$ is also uniquely estimated.

3.2.4 Hypothesis of interest

The analysis of longitudinal data is usually carried out when the value of matrix $\mathbf{B}$ is known that $\mathbf{B} \neq \mathbf{0}$, the hypothesis of the form (3.25) can be tested under the given model,

$$H_0 : \mathbf{FBG} = \mathbf{0} \text{ } versus \text{ } H_1 : \mathbf{B} \text{ } unrestricted \text{ }, \tag{3.25}$$

where $\mathbf{F}$ and $\mathbf{G}$ are known matrices and $\mathbf{0}$ is the null matrix. It is noted that each matrix involved in the null hypothesis (3.25) represent a specific quantity of interest [32]. These matrices are selected appropriately depending on the parameters of interest that they are needed to test, for instance, slopes of the growth curve or any other coefficients of time. In this work, the likelihood ratio test statistic proposed by [27] and [22] is used to test the trends (i.e degrees) of the model and to estimate the equality of the growth curves in all k treatment groups.

3.2.5 The likelihood ratio testing, $H_0 : \mathbf{FBG} = \mathbf{0}$, in the GCM

In this subsection, the Likelihood ratio test statistic for testing bilinear unrestricted in the GCM will be presented. The concept of covariance matrix $\mathbf{\Sigma}$ will be taken into consideration. The **likelihood ratio test** as follows in book [38].

Definition 3.2 *the book Let $(Y_1, \dots, Y_n)$ be the data with joint pdf or joint pmf $f(y, \theta)$ where θ is a vector of unknown parameters with parameter space Θ. Let $\widehat{\theta}$ be the Maximum Likelihood Estimator (MLE) of θ and let $\widehat{\theta}_0$ be the MLE of θ if parameter space Θ_0 (where $\Theta_0 \subset \Theta$). A likelihood ratio test (LRT) statistic for testing $H_0 : \theta \in \Theta_0$ versus $H_1 : \theta \in \Theta_0^c$ is*

$$\lambda(y) = \frac{\mathbf{L}(\widehat{\theta}_0, y)}{\mathbf{L}(\widehat{\theta}, y)} = \frac{sup_{\Theta_0}\mathbf{L}(\theta, y)}{sup_{\Theta}\mathbf{L}(\theta, y)}.$$

*The **likelihood ratio test** (LRT) has a rejection region R of the form*

$$R = \{y; \lambda(y) \leq c\},$$

where $0 \leq c \leq 1$, and $\alpha = sup_{\theta_0 \in \Theta_0} P_\theta(\lambda(\mathbf{Y}) \leq c)$. Suppose $\theta_0 \in \Theta_0$ and $sup_{\theta_0 \in \Theta_0} P_\theta(\lambda(\mathbf{Y}) \leq c) = P_{\theta_0}(\lambda(\mathbf{Y}) \leq c)$. Then $\alpha = P_{\theta_0}(\lambda(\mathbf{Y}) \leq c)$.

Under the full rank conditions, Khatri C.G provided maximum likelihood estimates for $\mathbf{B}$ and $\mathbf{\Sigma}$ in the GCM as we have shown in the equations (3.21) and (3.22) respectively. He also provided Likelihood ratio test statistic for testing the general linear hypothesis for the GCM which was considered by Patthoff and Roy [26] as an important contribution, because it avoids the use of an arbitrary matrix $\mathbf{G}$. Despite theoretical advances for the GMANOVA model that allowed researchers to provide MLE estimators and likelihood ratio tests, practical applications using the model remain limited. von Rosen provided the practical application based on testing the trends and the estimation of the equality of the curves in k treatment groups by using likelihood ratio test statistic in its book [32]. In this thesis, the test of hypothesis of the form (3.25) can be split into 2 tests.

i. If (3.25) is to be the hypothesis that all k growth curves are actually of degree $(q-1)$ or less, we set $\mathbf{G} = \mathbf{I}$, and take $\mathbf{F}$ to be a $1 \times q-1$ vector with all 0's except for a 1 as the last element. This test is based on testing of trends of the GCM.

ii. If (3.25) is to be the hypothesis that all k growth curves are equal except possibly for the additive constant β_{1k}, we take $\mathbf{F}$ to be a $q \times (q-1)$ matrix whose first column contains all 0's and whose last $(q-1)$ columns constitute the identity matrix, and $\mathbf{C}$ is taken to be $k \times (k-1)$ bidiagonal matrix whose the main diagonal entries are all equal to one and entries below the main diagonal are all equal to minus one.

For further understanding, consider the following example,

Example 3.1 *For the GCM defined in* (3.1)*, suppose that*

$$\mathbf{A} = \begin{pmatrix} 1 & t_1 & t_1^2 \\ 1 & t_2 & t_2^2 \\ \vdots & \vdots & \vdots \\ 1 & t_p & t_p^2 \end{pmatrix},$$

and

$$\mathbf{C} = (\boldsymbol{1}_{n_1}^{'} \otimes (1:0:0:0:0)^{'} : \boldsymbol{1}_{n_2}^{'} \otimes (0:1:0:0:0)^{'} : \boldsymbol{1}_{n_3}^{'} \otimes (0:0:1:0:0)^{'} \\ : \boldsymbol{1}_{n_4}^{'} \otimes (0:0:0:1:0)^{'} : \boldsymbol{1}_{n_5}^{'} \otimes (0:0:0:0:1)^{'}),$$

indicating that the degrees of the model is 2 *and there are* 5 *treatment groups.*

i. If one want to test the quadratic growth curve model, then

$$H_0 : \boldsymbol{FB} = \mathbf{0} \; versus H_1 : \mathbf{B} \; unrestricted.$$

We choose $\mathbf{G} = \mathbf{I}$ *and* $\mathbf{F} = [0:0:1]$ *which is appropriated for the model.*

ii. If one want to test the equality of $k = 5$ *growth curves except possibly for the additive constant* β_{1k}*, then*

$$H_0 : \boldsymbol{FBG} = \mathbf{0} \; versus H_1 : \mathbf{B} \; unrestricted,$$

where

$$\mathbf{F} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

and

$$\mathbf{G} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & -1 \end{pmatrix}.$$

A common approach for obtaining tests based on the likelihood ratio is to obtain estimators under the hypothesis H_0 and under the alternative hypothesis H_1, which are inserted in the likelihoods corresponding to H_0 and H_1 [32]. Note that $\mathbf{FBG} = \mathbf{0}$ is equivalent to

$$\mathbf{B} = (\mathbf{F}^{'})^{o}\boldsymbol{\Theta}_1 + \mathbf{F}^{'}\boldsymbol{\Theta}_2\mathbf{G}^{o'}, \tag{3.26}$$

where $\mathbf{\Theta}_1$ and $\mathbf{\Theta}_2$ are the unknown new parameter matrices. The equation (3.26) is the reparametrization of $\mathbf{FBG} = \mathbf{0}$ and it is due to the fact that $\mathbf{G}^{o'}\mathbf{G} = \mathbf{0}$. After this reparametrization, under the H_0 and H_1, the model can equivalently be written as follows

$$H_1 : \mathbf{X} = \mathbf{ABC} + \mathbf{E}, \tag{3.27}$$

and

$$H_0 : \mathbf{X} = \mathbf{A}\left[(\mathbf{F}^{'})^o\Theta_1 + \mathbf{F}^{'}\mathbf{\Theta}_2\mathbf{G}^{o'}\right]\mathbf{C} + \mathbf{E},$$
$$H_0 : \mathbf{X} = \mathbf{A}(\mathbf{F}^{'})^o\mathbf{\Theta}_1\mathbf{C} + \mathbf{A}\mathbf{F}^{'}\mathbf{\Theta}_2\mathbf{G}^{o'}\mathbf{C} + \mathbf{E}.$$

Under H_1, the maximum Likelihood estimators of the mean ($\mathbf{ABC}$) and $\mathbf{\Sigma}$ are defined in the equation (3.24) and (3.22) respectively. According to [32], the maximum of the likelihood is proportional to

$$\left|\mathbf{S}_1 + \mathbf{P}^{'}_{\mathbf{A}^o,\mathbf{S}^{-1}}\mathbf{X}_0\mathbf{P}_{\mathbf{C}'}\mathbf{X}_0^{'}\mathbf{P}_{\mathbf{A}^o,\mathbf{S}^{-1}}\right|^{-\frac{n}{2}}, \tag{3.28}$$

and now $\mathbf{S}_1 = \mathbf{X}_0(\mathbf{I} - \mathbf{P}_{\mathbf{C}'})\mathbf{X}_0^{'}$, where it has been indicated that the observed values are used. The maximum Likelihood estimators of the mean and $\mathbf{\Sigma}$ under the restricted model (i.e, H_0) are given as follows:

$$\mathbf{A}\widehat{\mathbf{B}}\mathbf{C} = \mathbf{A}(\mathbf{F}^{'})^o\widehat{\mathbf{\Theta}}_1\mathbf{C} + \mathbf{A}\mathbf{F}^{'}\widehat{\mathbf{\Theta}}_2\mathbf{G}^{o'}\mathbf{C} = \mathbf{P}^{'}_{\mathbf{A}_1,\mathbf{S}_1}\mathbf{X}\mathbf{P}_{\mathbf{C}_1{}'} + \mathbf{P}_{\widehat{\mathbf{Q}}_1,\mathbf{A}_2,\widehat{\mathbf{S}}_2}\mathbf{X}\mathbf{P}_{\mathbf{C}'{}_2}, \tag{3.29}$$

and

$$n\widehat{\mathbf{\Sigma}} = (\mathbf{X} - \mathbf{A}\widehat{\mathbf{B}}\mathbf{C})(\mathbf{X} - \mathbf{A}\widehat{\mathbf{B}}\mathbf{C})^{'} = \widehat{\mathbf{S}}_2 + \widehat{\mathbf{Q}}_2^{'}\widehat{\mathbf{Q}}_1^{'}\mathbf{X}\mathbf{P}_{\mathbf{C}_2^{'}}\mathbf{X}^{'}\widehat{\mathbf{Q}}_1\widehat{\mathbf{Q}}_2. \tag{3.30}$$

where

$$\begin{aligned}\mathbf{S}_1 = \mathbf{X}_0(\mathbf{I} - \mathbf{P}_{\mathbf{C}'})\mathbf{X}^{'}{}_0, \quad \mathbf{C}_1 = \mathbf{C}, \quad \mathbf{C}_2 = \mathbf{G}^{o'}\mathbf{C}, \quad \mathbf{A}_1 = \mathbf{A}(\mathbf{F}^{'})^o,\\
\mathbf{A}_2 = \mathbf{A}\mathbf{F}^{'}, \quad \widehat{\mathbf{Q}}_1 = \mathbf{I} - \mathbf{P}^{'}{}_{A_1,S_1},\\
\widehat{\mathbf{Q}}_2 = \mathbf{I} - \mathbf{P}^{'}{}^{'}_{\mathbf{P}\widehat{\mathbf{Q}}_1^{'},\mathbf{A}_2,\widehat{\mathbf{S}}_2},\\
\widehat{\mathbf{Q}}_2^{'}\widehat{\mathbf{Q}}_1^{'} = \mathbf{I} - \mathbf{P}^{'}{}_{A_1,S_1} - \mathbf{P}^{'}{}_{\widehat{Q}_1^{'},A_2,\widehat{S}_2},\\
\widehat{\mathbf{S}}_2 = \mathbf{S}_1 + \widehat{\mathbf{Q}}_1^{'}\mathbf{X}(\mathbf{P}^{'}_{\mathbf{C}_1} - \mathbf{P}^{'}_{\mathbf{C}_2})\mathbf{X}^{'}\widehat{\mathbf{Q}}_1.\end{aligned}$$

And also, von Rosen (2018) shows that the maximum of the likelihood under H_0 is proportional to

$$\left|\widehat{\mathbf{S}}_2 + \widehat{\mathbf{Q}}_2^{'}\widehat{\mathbf{Q}}_1^{'}\mathbf{X}_0\mathbf{P}_{\mathbf{C}_2^{'}}\mathbf{X}_0^{'}\widehat{\mathbf{Q}}_1\widehat{\mathbf{Q}}_2\right|^{-\frac{n}{2}}. \tag{3.31}$$

Let $\widehat{\mathbf{\Sigma}}_{H_0}$ be the MLE of $\mathbf{\Sigma}$ under the null hypothesis defined in (3.30) and let $\widehat{\mathbf{\Sigma}}_{H_1}$ denote the the MLE of $\mathbf{\Sigma}$ under the alternative hypothesis defined in (3.27), then the likelihood ratio for testing these hypotheses is

$$\lambda_0^{\frac{2}{n}} = \frac{\left|\widehat{\mathbf{\Sigma}}_{H_0}\right|}{\left|\widehat{\mathbf{\Sigma}}_{H_1}\right|} = \frac{\left|\widehat{\mathbf{S}}_2 + \widehat{\mathbf{Q}}_2^{'}\widehat{\mathbf{Q}}_1^{'}\mathbf{X}_0\mathbf{P}_{\mathbf{C}_2^{'}}\mathbf{X}_0^{'}\widehat{\mathbf{Q}}_1\widehat{\mathbf{Q}}_2\right|}{\left|\mathbf{S}_1 + \mathbf{P}^{'}_{\mathbf{A}^0,S_1^{-1}}\mathbf{X}_0\mathbf{P}_{\mathbf{C}_1^{'}}\mathbf{X}_0^{'}\mathbf{P}_{\mathbf{A}^0,S_1^{-1}}\right|}. \tag{3.32}$$

This expression appears to be difficult to handle. That is why, the expression (3.32) can be transformed into a well known form of a ratio of determinants of Wishart distribution variables [32]. So, $\lambda^{\frac{2}{n}}$ follows the same distributions as

$$\frac{|\mathbf{V}+\mathbf{U}|}{|\mathbf{V}|}. \tag{3.33}$$

where $\mathbf{V}$ and $\mathbf{U}$ are independent, with $\mathbf{V} \sim W_{r(\mathbf{M})}(\mathbf{I}, n - r(\mathbf{C}) - p + r(\mathbf{A}))$ and under H_0, $\mathbf{U} \sim W_{r(\mathbf{M})}(\mathbf{I}, r(\mathbf{N}))$ with $\mathbf{M}$ is any matrix of full rank such that $C(\mathbf{M}) = C(\mathbf{A}^{'}) \cap C(\mathbf{F}^{'})$ and $\mathbf{N}$ is any matrix of full rank such that $C(\mathbf{N}) = C(\mathbf{C}) \cap C(\mathbf{G})$. The matrices $\mathbf{M}$ and $\mathbf{N}$ are assumed to be different from zero matrix. If one of them is zero, the $\lambda_0 = 1$ i.e the hypothesis becomes meaningless to test. Therefore, the likelihood ratio test statistic defined in equation (3.32) is equivalent to $\mathbf{U}_{p,m,f}^{-\frac{2}{n}}$

$$U_{p,m,f} = \frac{\left|\widehat{\mathbf{\Sigma}}_{H_1}\right|}{\left|\widehat{\mathbf{\Sigma}}_{H_0}\right|},$$

and $f = n - r(\mathbf{C}) - p + r(\mathbf{A})$ and $m = \dim\{C(\mathbf{G}) \cap C(\mathbf{C})\}$ [32]. Theorem 3.2, 3.4 and Corollary 3.3 which are found in [32] will be considered in order to decide whether the null hypothesis will be rejected or not.

Theorem 3.2 *For the growth curve model presented in Definition* (3.1)*, the null hypothesis* $H_0 : \mathbf{FBG} = \mathbf{0}$ *against an alternative without restrictions on* $\mathbf{B}$*. Let* λ_0 *be the observed value of* λ*, given in* (3.32)*,and let*

$$t_0 = \frac{2}{n}(f - \frac{1}{2}(p_0 - m + 1)) \ln \lambda_0,$$

where $f = n - r(\mathbf{C}) - p + r(\mathbf{A})$*,* $p_0 = \dim\left\{C(\mathbf{F}^{'}) \cap C(\mathbf{A}^{'})\right\}$ *and* $m = \dim\{C(\mathbf{G}) \cap C(\mathbf{C})\}$*. The likelihood ratio test, approximately at significance level* α*, rejects the hypothesis if* t_0 *satisfies*

$$\begin{aligned} P\left\{\chi^2_{p_0m} \geq t_0\right\} + c_1(1-c_1)(P\left\{\chi^2_{p_0m+4} \geq t_0\right\} - P\left\{\chi^2_{p_0m} \geq t_0\right\}) \\ +c_2(P\left\{\chi^2_{p_0m+8} \geq t_0\right\} - P\left\{\chi^2_{p_0m} \geq t_0\right\}) \leq \alpha, \end{aligned}$$

where

$$\begin{aligned} c_1 &= \frac{p_0m(p_0^2 + m^2 - 5)}{48(f - \frac{1}{2}(p_0 - m + 1))^2}, \\ c_2 = \frac{1}{2}c_1^2 + &\frac{p_0m(3p_0^4 + 3m^4 + 10p_0^2m^2 - 50(p_0^2 + m^2) + 159)}{1920(f - \frac{1}{2}(p_0 - m + 1))^4}. \end{aligned}$$

Corollary 3.3 *If* $\mathbf{G} = \mathbf{I}$*, for the growth curve model, presented in definition* (3.1)*, the test of the null hypothesis* $\mathbf{FB} = \mathbf{0}$ *against an alternative without restriction is obtained from Theorem* (3.2) *if one uses* $m = r(\mathbf{C})$*.*

Theorem 3.4 *The model and hypothesis are the same as those in Theorem* (3.2)*. Let* λ *be given in* (3.32) *and* $\mathbf{U}_{p_0,m,f} = \lambda^{-\frac{2}{n}}$*, where* $p_0 = \dim\left\{C(\mathbf{F}^{'}) \cap C(\mathbf{A}^{'})\right\}$*,* $f = n - r(\mathbf{C}) - p + r(\mathbf{A})$ *and* $m = \dim\{C(\mathbf{G}) \cap C(\mathbf{C})\}$*. Then* *i. if* $p_0 = 2$,

$$T_{11} = \frac{(f-1)}{m}(1 - U_{2,m,f}^{\frac{1}{2}})/U_{2,m,f}^{\frac{1}{2}} \sim F_{2m,2(f-1)}; \tag{3.34}$$

ii. if $p_0 = 1$,

$$T_{12} = \frac{f}{m}(1 - U_{1,m,f})/U_{1,m,f} \sim F_{m,f}. \tag{3.35}$$

Theorem 3.4 and Corollary 3.3 will be used to test the trends or curves of the wilting rate of rose flowers in all Calcium foliar feeds. Also, Theorem 3.4 will be used to test if the wilting rate of rose flowers will be the same or not across the time in the 5 different concentration of Calcium foliar feed. In addition, according the above Theorem and Corollary 3.3, the null hypothesis is rejected at significance level of 0.05 if $T_{11} > F_{2m,2(f-1)}$ or $T_{12} > F_{m,f}$.

Chapter 4

RESULTS AND DISCUSSIONS

In this section, we return to the problem of analyzing the given data in order to achieve the objectives of the study. After executing Matlab commands and functions which described in chapter 3, section 3.1, the results of Exploratory Data Analysis, inferential analysis and fitted trends in the GCM are obtained. Recall that the average of wilting scores of rose flowers for each time within the groups is considered as the **wilting rate** of rose flowers and concentration of Calcium foliar feed is referred as foliar feed.

4.1 Exploratory Data Analysis

Exploratory analysis of longitudinal data seeks to discover patterns of systematic variation across treatment groups, as well as aspects of random variation that distinguish individual observations. It is interested in summary statistics such as group means and Standard Error of the mean over time in order to investigate whether different groups are changing in a similar or different way by time of measurements.

4.1.1 Descriptive statistics

Descriptive Statistics is a way of giving a brief overview of the dataset which will be interested in the study and it is a helpful way to understand characteristics of your data and to get a quick summary of it. Recall that the longitudinal (or more general repeated measurement data) situation involves observations of the same response repeatedly over time (or some other condition) for each of a number of treatment groups. So, the data of the wilting scores of rose flowers is measured on each individual at 4 different time points t_1, t_2, t_3, t_4 =day 0, day 3, day 6, day 9. Tables 4.1 and 4.2 generate group means and the standard error of the mean over time respectively, where the standard error of the mean over time measures the dispersion of sample means around the population mean and it is equal to the sample standard deviation (often SD) divided by the square root of sample size.

Table 4.1 and 4.2 show that the wilting rate of rose flowers at day 0 is the same in all foliar feeds i.e the mean is one in all foliar feeds and there is a least wilted. This is expected since the measurements is directly done at the time of applying the foliar

Table 4.1: The mean of wilting scores of rose flowers over time

Foliar feeds	Group means over time			
	day 0	day 3	day 6	day 9
1	1.0000	1.0460	1.1381	1.4495
2	1.0000	1.0000	1.1841	1.4388
3	1.0000	1.0000	1.2301	1.4495
4	1.0000	1.0000	1.3682	1.4848
5	1.0000	1.0000	1.4035	1.6856

Table 4.2: Standard error (SE) of the mean of the wilting scores of rose flowers over time

Foliar feeds	SE of the mean over time			
	day 0	day 3	day 6	day 9
1	0	0.0460	0.0690	0.0353
2	0	0	0.0728	0.0715
3	0	0	0.0728	0.0353
4	0	0	0.0460	0.0467
5	0	0	0.0614	0.0770

feeds. The impact foliar feed 1 on the wilting scores of rose flowers can be seen by the mean at the day 3 visit i.e the wilting rate of rose flowers increases slowly. The wilting rate of rose flowers increases over time from day 6 of visiting for all foliar feeds. In the foliar feed 1, foliar feed 2, foliar feed 3 foliar feed 4 and foliar feed 5, the means and standard error (SE) of the mean of the wilting scores of rose flowers at day 6 are 1.1381($S.E = 0.0690$), 1.1841($S.E = 0.0728$), 1.2301($S.E = 0.0728$), 1.3682($S.E = 0.0460$) and 1.4035($S.E = 0.0614$) up slightly from the day 0 mean of 1($S.E = 0$), respectively. This summarize that all foliar feeds are significantly different at day 6. As the days of visiting increase, the further change is observed. The foliar feed 1, foliar feed 2, foliar feed 3 foliar feed 4 and foliar feed 5 have the means that increase to 1.4495, 1.4388, 1.4495, 1.4848 and 1.6856, respectively, at the day 9. Note that the increase in average of wilting scores of rose flowers is larger than the others for day 6 and 9 in the foliar feeds 5. The above summaries suggest that the foliar feeds have significant effect on the wilting scores of rose flowers over time.

4.1.2 Profile plots

Profile plots provide another useful graphical summary of the data. In Figure 4.1, the sample mean for each group are plotted against the time of measurements. Figure 4.1 is a profile plot of the growth curves. In this figure, the x-axis represents the time of measurement varies from day 1 up to day 4 and the y-axis represents the wilting rate of rose flowers across the time in the groups. That is, the dependent variable is the wilting rate of rose flowers and the independent variable is time. In addition, group 1, group 2, group 3, group 4 and group 5 represent the concentration of Calcium foliar feed of level 1, level 2, level 3, level 4 and level 5, respectively. We observe that in Figure 4.1, the growth in all foliar feeds (levels) have the same trend or growth curve and it is approximately modeled with a second order polynomial.

Figure 4.1 also shows that the foliar feed 5 produces the largest average wilting score while foliar feed 2 has the least average score. It is observed in Figure 4.1 that at day 0, all the wilting rate are equal and the rate of change of wilting score is slower between day 0 and day 3 than between day 6 and day 9, that is, wilting process is more observed between day 6 and day 9. Figure also shows that the wilting rate of rose flowers in all groups increases as time of measurements increases.

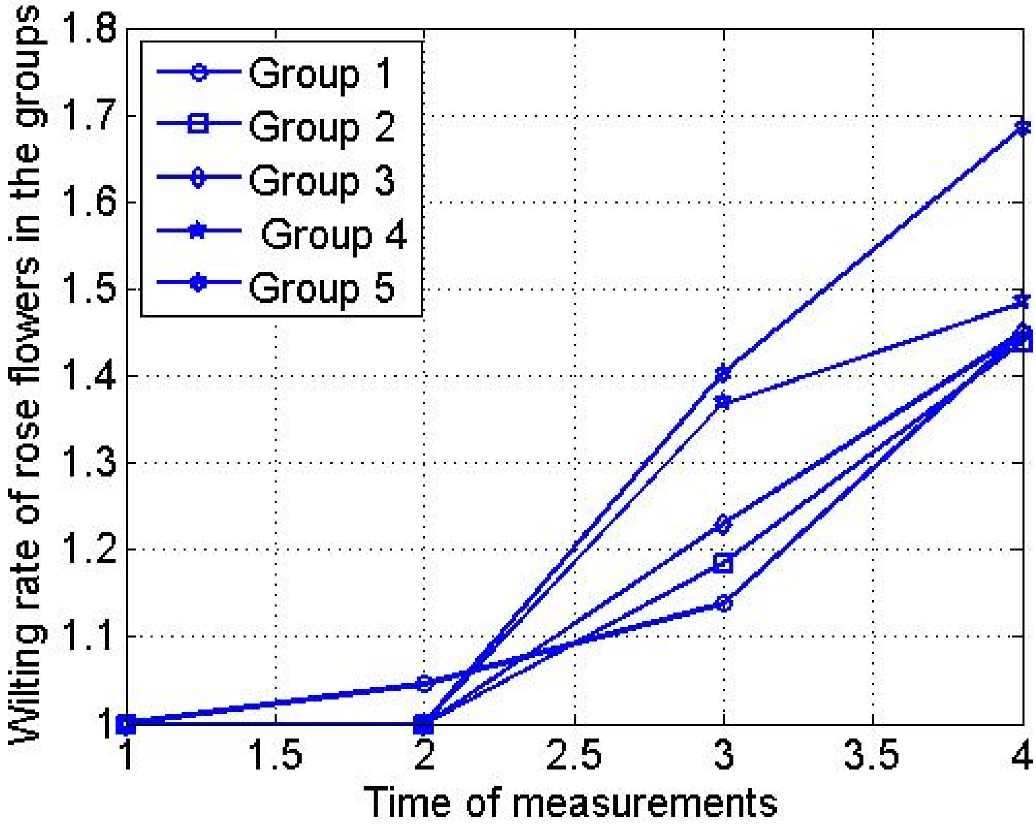

Figure 4.1: The group means of the wilting scores of rose flowers by time

4.2 Inferential analysis

In this section, we construct a model which describes the data. Consider the longitudinal data of wilting scores of rose flowers described on descriptive statistic. To generate the test presented in chapter 3, section 3.2, the model (3.1), $\mathbf{X} = \mathbf{ABC}+\mathbf{E}$ is supposed to be valid with

$$\mathbf{A} = \begin{pmatrix} 1 & t_1 & t_1^2 \\ 1 & t_2 & t_2^2 \\ 1 & t_3 & t_3^2 \\ 1 & t_4 & t_4^2 \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 \\ 1 & 2 & 4 \\ 1 & 3 & 9 \\ 1 & 4 & 16 \end{pmatrix},$$

and $\mathbf{C} = (\mathbf{1}_{n_1}^{'} \otimes (1:0:0:0:0)^{'} : \mathbf{1}_{n_2}^{'} \otimes (0:1:0:0:0)^{'} : \mathbf{1}_{n_3}^{'} \otimes (0:0:1:0:0)^{'} : \mathbf{1}_{n_4}^{'} \otimes (0:0:0:1:0)^{'} : \mathbf{1}_{n_5}^{'} \otimes (0:0:0:0:1)^{'})$.
According to the second order on time in the within-individuals design matrix $\mathbf{A}$,

we can say that the growth is modeled with a second-order polynomial but the test is needed. So, we need to examine whether the growth curves due to the 5 foliar feeds are quadratic or not by testing whether the coefficients of the second-order terms in **ABC** are zero. The following hypotheses can be formulated

$$H_0 : \mathbf{FB} = \mathbf{0} \textit{ versus } H_1 : \mathbf{B} \textit{ unrestricted}, \tag{4.1}$$

where

$$\mathbf{F} = \begin{pmatrix} 0 & 0 & 1 \end{pmatrix}$$

and we set $\mathbf{G} = \mathbf{I}$
The test (4.1) is equivalent to

$$H_0 : \beta_{31} = \beta_{32} = \beta_{33} = \beta_{34} = \beta_{35} = 0$$

In this case, according to Theorem 3.2 and Corollary 3.3, $f = 39, p_0 = 1$ and $m = 5$ respectively. Since $p_0 = 1$, Theorem 3.4 (ii) can be applied. In addition, the calculations show that $\lambda_0^{\frac{2}{45}} = 2.2561$. Thus, $U_{1,5,39} = \lambda_0^{-\frac{2}{45}} = 0.443302$ and

$$T_{12} = \frac{39}{5}(1 - U_{1,5,39})/U_{1,5,39} = 9.795 > F_{0.05}(5, 39) = 2.25. \tag{4.2}$$

Hence, H_0 can be rejected at significance level of 0.05 since $T_{12} > F_{0.05}(5, 39)$. So, the estimated growth curves are modeled with a second order polynomial.
After fitting the GCM defined in equation (3.1), one often asks whether or not the quadratic growth curves in all foliar feeds are equal or identical. Recall that the unknown parameter matrix (**B**) consists of all coefficients of time include slope and quadratic coefficients. Also, it has intercept and it can be written as:

$$\mathbf{B} = \begin{pmatrix} \beta_{11} & \beta_{12} & \beta_{13} & \beta_{14} & \beta_{15} \\ \beta_{21} & \beta_{22} & \beta_{23} & \beta_{24} & \beta_{25} \\ \beta_{31} & \beta_{32} & \beta_{33} & \beta_{34} & \beta_{35} \end{pmatrix} = \begin{pmatrix} \boldsymbol{\beta}_1 & \boldsymbol{\beta}_2 & \boldsymbol{\beta}_3 & \boldsymbol{\beta}_4 & \boldsymbol{\beta}_5 \end{pmatrix}. \tag{4.3}$$

The null hypothesis for testing if the wilting rate of rose flowers are the same or not across the time for 5 groups can be formulated as follows:

$$\begin{aligned} H_0 &: \beta_{11} = \beta_{12} = \beta_{13} = \beta_{14} = \beta_{15}, \\ &: \beta_{21} = \beta_{22} = \beta_{23} = \beta_{24} = \beta_{25}, \\ &: \beta_{31} = \beta_{32} = \beta_{33} = \beta_{34} = \beta_{35}. \end{aligned} \tag{4.4}$$

This hypothesis is equivalent to

$$\begin{aligned} H_0 &: \beta_{11} - \beta_{12} = \beta_{12} - \beta_{13} = \beta_{13} - \beta_{14} = \beta_{14} - \beta_{15} = 0, \\ &: \beta_{21} - \beta_{22} = \beta_{22} - \beta_{23} = \beta_{23} - \beta_{24} = \beta_{24} - \beta_{25} = 0, \\ &: \beta_{31} - \beta_{32} = \beta_{32} - \beta_{33} = \beta_{33} - \beta_{34} = \beta_{34} - \beta_{35} = 0. \end{aligned} \tag{4.5}$$

The hypothesis in (4.5) can be written in matrix form:

$$H_0 : \mathbf{FBG} = \mathbf{0}.$$

where

$$\mathbf{F} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

and

$$\mathbf{G} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 \\ 0 & -1 & 1 & 0 \\ 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & -1 \end{pmatrix}.$$

Since, this work interest in studying the effects of time, there is no need to consider the intercept $(\beta_{1k}), k = 1, \ldots, 5$. So testing intercept terms seem redundant and useless. The null hypothesis defined on (4.4) can be reduced to

$$\begin{aligned} H_0 &: \beta_{21} = \beta_{22} = \beta_{23} = \beta_{24} = \beta_{25}, \\ &: \beta_{31} = \beta_{32} = \beta_{33} = \beta_{34} = \beta_{35}. \end{aligned}$$

In this case, matrix $\mathbf{F}$ can be transformed into

$$\mathbf{F} = \begin{pmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix},$$

Moreover, according to Theorem 3.2 and Corollary 3.3, $f = 39, p_0 = 2$ and $m = 5$, respectively. Since $p_0 = 2$, Theorem 3.4 (i) can be applied instead of using Theorem 3.2. In addition, the calculations yield $\lambda_0^{\frac{2}{45}} = 1.9356$. Thus, $U_{2,5,39} = \lambda_0^{-\frac{2}{45}} = 0.5166$ and

$$T_{11} = \frac{38}{5}(1 - U_{2,5,39}^{\frac{1}{2}})/U_{2,5,39}^{\frac{1}{2}} = 2.9739 > F_{0.05}(10, 76) = 1.9577; \qquad (4.6)$$

Hence, H_0 is rejected at significance level of 0.05 as $T_{11} > F_{0.05}(10, 76)$. So, we conclude that the wilting rate of rose flowers are different in all foliar feeds, this means that the quadratic growth curves for the five foliar feeds are not the same or identical. That is, the foliar feed has significant effects on the wilting process of rose flowers over time.

4.3 Fitted trends in the GCM

After estimating the appropriate order of the GCM, we estimate parameters ($\mathbf{B}$ and $\mathbf{\Sigma}$). For this case, the maximum likelihood estimator is preferred as method of parameters estimation because this method is very important for large sample also its asymptotic behavior is the best. This method was also discussed on chapter 3 in 3.2.3 subsection. Therefore, after some computations, we observe that under the model (3.1), the MLE of the matrix of unknown parameters $\mathbf{B}$ is given by

$$\widehat{\mathbf{B}} = \begin{pmatrix} 1.0196 & 1.0563 & 1.0444 & 1.0096 & 1.0544 \\ -0.0430 & -0.0848 & -0.0699 & -0.0263 & -0.0976 \\ 0.0422 & 0.0473 & 0.0442 & 0.0354 & 0.0620 \end{pmatrix},$$

and the MLE of $\mathbf{\Sigma}$ is obtained as

$$\widehat{\mathbf{\Sigma}} = \begin{pmatrix} 0.0005 & 0.0002 & 0.0019 & -0.0004 \\ 0.0002 & 0.0035 & 0.0027 & 0.0024 \\ 0.0019 & 0.0027 & 0.0493 & 0.0128 \\ -0.0004 & 0.0024 & 0.0128 & 0.0256 \end{pmatrix}.$$

The estimated mean growth curves defined in equation (3.7) in all foliar feeds become

$$\begin{aligned}
\widehat{\mu}_1(t) &= 1.0196 - 0.0430t + 0.0422t^2, \\
\widehat{\mu}_2(t) &= 1.0563 - 0.0848t + 0.0473t^2, \\
\widehat{\mu}_3(t) &= 1.0444 - 0.0699t + 0.0442t^2, \\
\widehat{\mu}_4 C &= 1.0096 - 0.0263t + 0.0354t^2, \\
\widehat{\mu}_5(t) &= 1.0544 - 0.0976t + 0.0620t^2.
\end{aligned}$$

The fitted growth curves are plotted in Figure 4.2 where t is time of measurements and $\widehat{\mu}_1(t), \widehat{\mu}_2(t), \widehat{\mu}_3(t), \widehat{\mu}_4(t)$ and $\widehat{\mu}_5(t)$ represent the estimated mean growth curves for foliar feed 1 (group 1), foliar feed 2 (group 2), foliar feed 3 (group 3), foliar feed 4 (group 4) and foliar feed 5 (group 5), respectively. We observe that the estimated growth curves plot for each foliar feed represents the graph of second order polynomial. Figure 4.2 reveals that the foliar feed 5 produces the largest average wilting score while foliar feed 2 has the least average score. It also shows that at day 0, all the wilting scores are the same and the rate of change of wilting score is slower between day 0 and day 3 than between day 6 and day 9. That is, the wilting process is more observed between day 6 and day 9. Furthermore, the wilting rate of rose flowers in all groups increase as time of measurements increase. .

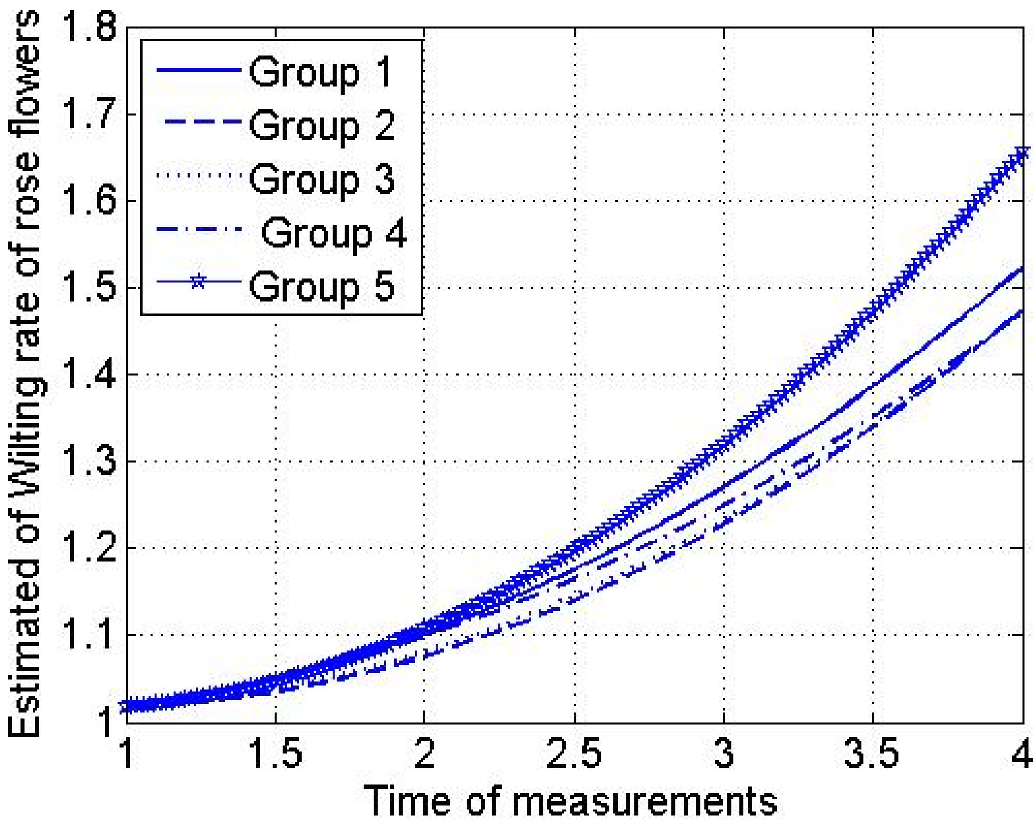

Figure 4.2: Fitted growth curves for five foliar feeds

Chapter 5

CONCLUDING REMARKS

This chapter focuses on the summary of contributions of this thesis and recommendation for future research.

In this thesis, the growth curve model for analyzing the effect of Calcium foliar feed on the wilting rate of rose flowers over time is presented. We first formulated the model followed by finding the MLEs for mean parameter $\mathbf{B}$ and covariance matrix $\mathbf{\Sigma}$. This work also applied LRT statistic for testing the trends or curves of the wilting rate of rose flowers in all foliar feeds. It is also used to investigate whether the wilting rate of rose flowers is the same or not across the foliar feeds over time.

The results reveal that the trends in all foliar feeds are estimated by the second order polynomial. The fitted growth curves plot for five foliar feeds by time in Figure 4.2 shows that the trends or curves are modeled with second order polynomial. The results also reveal that the foliar feed 5 produces the highest average wilting score while foliar feed 2 has the least average score. It is observed in Figure 4.2 that at day 0, all the wilting scores are the same. Figure 4.2 also shows that the rate of change of wilting score is slower between day 0 and day 3 than between day 6 and day 9, that is, wilting process is more observed between day 6 and day 9. Figure also shows that the wilting rate of rose flowers in all groups increase as time of measurements increase. The results also show that the wilting rate of rose flowers are not the same in all foliar feeds, that is, the growth curves are not all equal. This indicates a difference in wilting rate of rose flowers of the foliar feeds and the foliar feed has significant effects on the wilting process of rose flowers over time.

Therefore, growth curve model is an important tool for experimental research longitudinal data because this model was able to take into account the patterns of change of wilting rate over time. Since the wilting rate of rose flowers are not the same in all foliar feeds, this work recommends the future research to use subgroup analysis model in order to identity which treatment will perform better over time. This model can also assess the equality of certain treatment groups and might lead to identify the least or the most wilted over time when different concentration of Calcium foliar feeds are applied on rose flowers.

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