

## Lawley-Hotelling Test with One –Way Multivariate Repeated Measurements Analysis Of variance Model

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### Abstract

This research is devoted to the study the Lawley-Hotelling Test in One -Way Multivariate repeated measurements analysis of variance model (MRM ANOVA), which contains one between-units factor (group with  $q$  levels) incorporating one random effect, one within-units factor (time with  $p$  levels). The test statistics of various hypotheses on between-unites factor, within-units factor and the interaction between them are given. An application research, a study has been taken to diagnostics and isolation for kinds of becteria which compain with tissue cultivation for the dates and the study of frustrate affection for three kinds of extractor plant, which are called Rhus coriaria and cinnamomum zeylanicum, the excretes of adhesive for the Bswellia Sp plant and by using four kinds of solvent and three different condense. An experimental has been made for getting measurement for the best reacting extractor plant with the solvent by using different affection on frustrate core

**Keywords :**One-Way Multivariate Repeated Measures Model, Lawley-Hotelling test, Wishart distribution, MANOVA.

### الملخص:

هذا البحث مخصص لدراسة اختبار (Lawley-Hotelling) تحليل التبيان للقياسات المتكرره المتعدده ذات الاتجاه الواحد والذي يحتوي على عامل واحد بين الوحدات (مجموعه من  $q$  من المستويات مع تأثير عشوائي واحد ومعامل واحد بين الوحدات (وقت من  $p$  مستويات). كما تمت دراسة الاحصائيات الاختباريه للفرضيات المتعدده على معامل بين الوحدات ومعامل داخل الوحدات والتفاعل بينهما. وكدراسه تطبيقيه تم اعتماد بيانات تشخيص وعزل انواع من البكتريا .

### 1. Introduction

Repeated measurements analysis is widely used in many fields, for example, in the health and life sciences, epidemiology, biomedical, agricultural, industrial, psychological, educational research and so on. Repeated measurements is a term used to describe data in which the response variable for each experimental unite is observed on multiple occasions and possibly under different experimental conditions [9]. Repeated measures designs involving two or more independent groups are among the most common experimental designs in a variety of research settings. Various statistical procedures have been suggested for analyzing data from split-plot designs when parametric model assumptions are violated[6]. Repeated measurements analysis of variance, often referred to as randomized block and split-plot designs [5] and [8].

The focus of this paper is to study the one-way multivariate repeated measurements analysis of variance model (MRM ANOVA) . The test statistics of various hypotheses on between-units factors which are called the multivariate Lawley-Hotelling tests are given. The practical side of this paper is about tissue agriculture of Date palm trees. The most

important reasons that make us select the subject of Palm trees are the date fruit is considered as the most important commercial crops in the countries of the Middle East. And The dates contain high percentage of Antioxidants, which are important and necessary for the body. Among the wide horizon of Phenolic compounds, the dates contain PSinaPic acid, Coumaric Ferulic, as essential compounds. Antioxidants are paid a great attention by the specialists of nutrition science, and the researchers in the field of Medicine, because of its direct effect on the shorthand of the danger of chronic diseases, like cancer, heart diseases, aging and shocks[1]. The purpose of our study, having specified and separated three kinds of bacteria, is to examine the effect of the transactions of distance inhibitory of bacteria, and to know the best interaction of transactions through the application of the statistical test Lawly-Hotelling. The results of application are obtained by MATLAB (R 2012) program.

## 2- One –Way MRM Model

There is a variety of possibilities for the between- units factors in a one-way design. In a randomized one-way MRM experiment, the experimental units are randomized to one between-units factor (groups with  $q$  levels), one within-units factor (time with  $p$  levels) and random effect to experimental unit  $i$  within treatment group  $j$ , we define the following linear model and parameterization for the one-way repeated measurements design with one between- units factor :-

$$y_{ijk} = \mu + \tau_j + \delta_{i(j)} + \gamma_k + (\tau\gamma)_{jk} + e_{ijk} \quad \dots (2.1)$$

Where  $i = 1, \dots, n_j$  is an index for experimental unit within group  $j$ ,

$j = 1, \dots, q$  is an index for levels of the between-units factor (Group),

$k = 1, \dots, p$  is an index for levels of the within-units factor (Time),

$Y_{ijk} = [Y_{ijk1}, \dots, Y_{ijkp}]'$  is the response measurement at time  $k$  for unit  $i$  within group  $j$ ,  
 $\mu = [\mu_1, \dots, \mu_p]'$  is the overall mean,

$\tau_j = [\tau_{j1}, \dots, \tau_{jr}]'$  is the added effect for treatment group  $j$ ,

$\delta_{i(j)} = [\delta_{i(j)1}, \dots, \delta_{i(j)r}]'$  is the random effect due to experimental unit  $i$  within treatment group  $j$ ,

$\gamma_k = [\gamma_{k1}, \dots, \gamma_{kr}]'$  is the added effect for time  $k$ ,

$(\tau\gamma)_{jk} = [(\tau\gamma)_{jk1}, \dots, (\tau\gamma)_{jkr}]'$  is the added effect for the group  $j \times$  time  $k$  interaction,  
 and  $e_{ijk} = [e_{ijk1}, \dots, e_{ijkp}]'$  is the random error on time  $k$  for unit  $i$  within group  $j$ .  
 For the parameterization to be of full rank, we imposed the following set of conditions

$$\sum_{j=1}^q \tau_j = 0 \quad , \quad \sum_{k=1}^p \gamma_k = 0 \quad ,$$

$$\sum_{j=1}^q (\tau\gamma)_{jk} = 0 \quad , \quad \text{for each } k = 1, \dots, p ;$$

$$\sum_{k=1}^p (\tau\gamma)_{jk} = 0 \quad \text{for each } j = 1, \dots, q, \quad \dots (2.2)$$

we assume that the  $e_{ijk}$ 's,  $\delta_{i(j)}$ 's are independent with

$$e_{ijk} = [e_{ijk1}, \dots, e_{ijk r}]' \sim \text{i. i. d. } N_r (0, \Sigma_e),$$

$$\delta_{i(j)} = [\delta_{i(j)1}, \dots, \delta_{i(j)r}]' \sim \text{i. i. d. } N_r (0, \Sigma_\delta), \quad \dots (2.3)$$

where  $\Sigma_e, \Sigma_\delta$  are  $r \times r$  positive definite matrices.

Let  $Y_{ij} = [Y_{ij1}, Y_{ij2}, \dots, Y_{ijp}]'$ ,

$$\text{that is } Y_{ij} = \begin{bmatrix} Y_{ij11} & Y_{ij21} & \dots & Y_{ijp1} \\ Y_{ij12} & Y_{ij22} & \dots & Y_{ijp2} \\ \vdots & \vdots & \ddots & \vdots \\ Y_{ij1r} & Y_{ij2r} & \dots & Y_{ijpr} \end{bmatrix}. \quad \dots (2.4)$$

The variance- covariance matrix of  $\vec{Y}_{ij}$  is denoted as  $\Sigma$ , where

$\vec{Y}_{ij} = \text{Vec}(Y_{ij})$ . The  $\text{Vec}(\cdot)$  operator creates a column vector from a matrix  $Y_{ij}$  by simply stacking the column vectors of  $Y_{ij}$  below one another. The variance- covariance matrix  $\Sigma$  of the model (2.1) satisfies the assumption of compound symmetry, i.e.

$$\Sigma = I_p \otimes \Sigma_e + J_p \otimes \Sigma_\delta$$

$$= \begin{bmatrix} \Sigma_e + \Sigma_\delta & \Sigma_\delta & \dots & \Sigma_\delta \\ \Sigma_\delta & \Sigma_e + \Sigma_\delta & \dots & \Sigma_\delta \\ \vdots & \vdots & \ddots & \vdots \\ \Sigma_\delta & \Sigma_\delta & \dots & \Sigma_e + \Sigma_\delta \end{bmatrix}, \quad \dots (2.5)$$

Where  $I_p$  denotes the  $p \times p$  identity matrix,  $J_p$  denotes  $p \times p$  matrix of one's and  $\otimes$  is the Kronecker product operation of two matrices.

$$e_{ij} = [e_{ij1}, \dots, e_{ijp}]' \sim \text{i. i. d. } N_{p \times r} (0, I_p \otimes \Sigma_e). \quad \dots (2.6)$$

### 3- Transforming the One-Way Multivariate Repeated Measurements Analysis of variance (ANOVA) Mode

In this section, we use an orthogonal matrix to transform the observations  $Y_{ijk}$  for

$i = 1, \dots, n_j, j = 1, \dots, q, k = 1, \dots, p$ . Let  $U^*$  be any  $p \times p$  orthogonal matrix. It is partitioned as follows:  $U^* =$

$$\left( p^{\frac{-1}{2}} j_p \quad U \right), \quad \dots (3.1)$$

where  $j_p$  denotes the  $p \times 1$  vector of one's,  $U$  is  $p \times (p - 1)$  matrix,  $U' j_p = 0$  and  $U' U = I_{p-1}$ .

Let  $Y_{ij}^* = Y_{ij} U^*$

$$Y_{ij}^* = [Y_{ij1}^*, Y_{ij2}^*, \dots, Y_{ijp}^*]' = [Y_{ij1}, Y_{ij2}, \dots, Y_{ijp}]' U^*$$

$$\begin{bmatrix} Y_{ij11}^* & \dots & Y_{ijp1}^* \\ \vdots & \ddots & \vdots \\ Y_{ij1r}^* & \dots & Y_{ijpr}^* \end{bmatrix} = \begin{bmatrix} Y_{ij11} & \dots & Y_{ijp1} \\ \vdots & \ddots & \vdots \\ Y_{ij1r} & \dots & Y_{ijpr} \end{bmatrix} \left[ p^{-\frac{1}{2}} J_p \quad U \right]. \dots (3.2)$$

So

$$\begin{aligned} \text{Cov}(\vec{Y}_{ij}^*) &= \text{Cov}(\overline{Y_{ij} U^*}) = \text{Cov} \left( (U^{*'} \otimes I_r) \vec{Y}_{ij} \right) \\ &= (U^{*'} \otimes I_r) \Sigma(U^* \otimes I_r) \dots (3.3) \\ &= (U^{*'} \otimes I_r) (I_p \otimes \Sigma_e + J_p \otimes \Sigma_\delta) (U^* \otimes I_r). \\ &= I_p \otimes \Sigma_e + U^{*'} J_p U^* \otimes I_r \Sigma_\delta I_r \end{aligned}$$

We can write the above in the following matrix form:

$$\text{Cov}(\vec{Y}_{ij}^*) = \begin{bmatrix} \Sigma_e + p(\Sigma_\delta) & 0 & \dots & 0 \\ 0 & \Sigma_e & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \Sigma_e \end{bmatrix} \dots (3.4)$$

**4-Analysis of variance (ANOVA) for the One-Way Multivariate Repeated Measurements Model**

In this section, we study the ANCOVA for the effects of between-units factor and within-units factor for the one-way RM model (2.1). Also, we give the null hypotheses which are concerned with these effects and the interaction between them, and the test statistics for them.

Now

$$Y_{ij}^* = Y_{ij} U^*$$

$$Y_{ij1}^* = Y_{ij} p^{-\frac{1}{2}} j_p$$

$$\therefore Y_{ij1}^* = \begin{bmatrix} Y_{ij11}^* \\ Y_{ij12}^* \\ \vdots \\ Y_{ij1r}^* \end{bmatrix} = \begin{bmatrix} \frac{1}{\sqrt{p}} \sum_{k=1}^p Y_{ijk1} \\ \frac{1}{\sqrt{p}} \sum_{k=1}^p Y_{ijk2} \\ \vdots \\ \frac{1}{\sqrt{p}} \sum_{k=1}^p Y_{ijk r} \end{bmatrix},$$

From (2.1), we obtain

$$\begin{aligned}
 Y_{ij1}^* &= p^{-\frac{1}{2}} \sum_{k=1}^p Y_{ijk} \\
 &= p^{-\frac{1}{2}} \sum_{k=1}^p (\mu + \tau_j + \delta_{i(j)} + \gamma_k + (\tau\gamma)_{jk} + e_{ijk}) \\
 &= p^{\frac{1}{2}}\mu + p^{\frac{1}{2}}\tau_j + p^{\frac{1}{2}}\delta_{i(j)} + p^{-\frac{1}{2}} \sum_{k=1}^p e_{ijk}
 \end{aligned}$$

$$Y_{ij1}^* = \mu^* + \tau_j^* + \delta_{i(j)}^* + e_{ij1}^* \quad \text{Then the set of vectors} \\
 (\mathbf{Y}_{111}^*, \dots, \mathbf{Y}_{n_1 11}^*), (\mathbf{Y}_{121}^*, \dots, \mathbf{Y}_{n_2 21}^*), \dots, (\mathbf{Y}_{1q1}^*, \dots, \mathbf{Y}_{n_q q1}^*)'$$

have mean vectors

$$X_1 = \sqrt{p}\mu + \sqrt{p}\tau_1$$

$$X_2 = \sqrt{p}\mu + \sqrt{p}\tau_2$$

⋮

$$X_q = \sqrt{p}\mu + \sqrt{p}\tau_q$$

respectively, and each of them has covariance matrix  $\Sigma_e + p\Sigma_\delta$ .

So, the null hypothesis of the same treatment effects is:

$$H_{01}: \tau_1^* = \dots = \tau_q^* = 0$$

and are equivalent to the null hypothesis for the same average vector

$$H_{01} = X_1 = X_2 = \dots = X_q = 0.$$

The ANOVA based on the set of transformed observations above, the  $Y_{ij1}^*$ 's provides the ANOVA for the between-units effects. This leads to the following form for the sum squares terms:

$SS_G, SS_{u(G)}$ , where

$$\begin{aligned}
 SS_G &= \sum_{j=1}^q n_j (\bar{Y}_{j1}^* - \bar{Y}_1^*)(\bar{Y}_{j1}^* - \bar{Y}_1^*)' \\
 SS_{u(G)} &= \sum_{j=1}^q \sum_{i=1}^{n_j} (Y_{ij1}^* - \bar{Y}_{j1}^*)(Y_{ij1}^* - \bar{Y}_{j1}^*)', \text{ where}
 \end{aligned}$$

$$\bar{Y}_{j1}^* = \frac{\sum_{i=1}^{n_j} Y_{ij1}^*}{n_j}, \quad \bar{Y}_1^* = \frac{\sum_{j=1}^q \sum_{i=1}^{n_j} Y_{ij1}^*}{n}.$$

$$\text{Thus } SS_G \sim W_r(q-1, p\Sigma_\delta + \Sigma_e)$$

$$SS_{u(G)} \sim W_r(n-q, p\Sigma_\delta + \Sigma_e),$$

where  $W_r$  denotes the multivariate-Wishart distribution.

The ANOVA based on the  $k^{th}$  set of transformed observations, the  $Y_{ijk}^*$ 's for each

$$k = 2, \dots, p, \text{ i.e. } Y_{ijk}^* = \begin{bmatrix} Y_{112}^* & Y_{113}^* & \dots & Y_{11p}^* \\ Y_{212}^* & Y_{213}^* & \dots & Y_{21p}^* \\ \vdots & \vdots & & \vdots \\ Y_{n_1 12}^* & Y_{n_1 13}^* & \dots & Y_{n_1 1p}^* \\ Y_{122}^* & Y_{123}^* & \dots & Y_{12p}^* \\ Y_{222}^* & Y_{223}^* & \dots & Y_{22p}^* \\ \vdots & \vdots & \dots & \vdots \\ Y_{n_2 22}^* & Y_{n_2 23}^* & \dots & Y_{n_2 2p}^* \\ \vdots & \vdots & \dots & \vdots \\ Y_{1q2}^* & Y_{1q3}^* & \dots & Y_{1qp}^* \\ \vdots & \vdots & \dots & \vdots \\ Y_{n_q q2}^* & Y_{n_q q3}^* & \dots & Y_{n_q qp}^* \end{bmatrix}$$

has the model  $Y_{ijk}^* = \sum_{k'=1}^p u_{kk'} Y_{ijk'}$

$$= \sum_k^p u_{kk'} (\mu + \tau_j + \delta_{i(j)} + \gamma_{k'} + (\tau\gamma)_{jk'} + e_{ijk'}) \dots (4.1)$$

Because the components of  $(\mathbf{U}_{k1}, \dots, \mathbf{U}_{kp})$  sum to zero for each  $k=1, \dots, p$  the  $k^{th}$  model in (2.12) is equivalent to  $Y_{ijk}^* = \gamma_k^* + (\tau\gamma)_{jk}^* + e_{ijk}^*$ , ... (4.2)

where  $[\gamma_2^*, \dots, \gamma_p^*] = [\gamma_1, \dots, \gamma_p]U$ .

It is clear that when  $\gamma_1 = \dots = \gamma_p = 0$  then  $\gamma_2^* = \dots = \gamma_p^* = 0$

In another way, while  $\sum_{k=1}^p \gamma_k = 0$

$$[0, \gamma_2^*, \dots, \gamma_p^*] = [\gamma_1, \dots, \gamma_p]U^*$$

$$\text{Then } [\gamma_1, \dots, \gamma_p] = [0, \gamma_2^*, \dots, \gamma_p^*]U^{*'}$$

which implies  $\gamma_2^* = \dots = \gamma_p^* = 0$

Therefore, the hypothesis  $H_{02}: \gamma_1 = \dots = \gamma_p = 0$  is equivalent to the hypothesis

$$H_{02}: \gamma_2^* = \dots = \gamma_p^* = 0.$$

Similarly for each j

$$[(\tau\gamma)_{j2}^*, \dots, (\tau\gamma)_{jp}^*] = [(\tau\gamma)_{j1}, \dots, (\tau\gamma)_{jp}]U$$

And the hypothesis  $H_{03}: (\tau\gamma)_{j1} = \dots = (\tau\gamma)_{jp} = 0$

be equivalent to the hypothesis

$$H_{03}: (\tau\gamma)_{j2}^* = \dots = (\tau\gamma)_{jp}^* = 0.$$

The ANOVA based on the set of transformed observations above,  $[Y_{ij2}^*, \dots, Y_{ijp}^*]$  provides the ANOVA for within-units effects. This leads to the following forms for the sum squares terms :

$$\text{where } \bar{Y}_{jk}^* = \frac{\sum_{i=1}^{n_j} Y_{ijk}^*}{n_j},$$

$$1) \quad SSE = \sum_{k=2}^p \sum_{j=1}^q \sum_{i=1}^{n_j} (Y_{ijk}^* - \bar{Y}_{jk}^*)(Y_{ijk}^* - \bar{Y}_{jk}^*)'$$

$$SSE \sim W_r((p-1)(n-q), \Sigma_e)$$

$$\text{where } \bar{Y}_k^* = \frac{\sum_{j=1}^q n_j \bar{Y}_{jk}^*}{n}, \quad k = 2, \dots, p$$

$$2) \quad SS_{G \times T} = \sum_{k=2}^p \sum_{j=1}^q n_j (\bar{Y}_{jk}^* - \bar{Y}_k^*)(\bar{Y}_{jk}^* - \bar{Y}_k^*)'$$

$$SS_{G \times T} \sim W_r((p-1)(q-1), \Sigma_e)$$

$$3) \quad SS_{\text{Time}} = \sum_{k=2}^p n (\bar{Y}_k^* (\bar{Y}_k^*))'$$

$$SS_{\text{Time}} \sim W_r((p-1), \Sigma_e)$$

## 5 - Lawly-Hotelling Test

The Lawly-Hotelling statistic is defined as  $U^{(s)} = \text{tr}(E^{-1}H)$  and is also known as Lawly-Hotelling's table gives upper percentage points of the test statistic  $\frac{VE}{VH} U^{(s)}$  where :

E= sum squares matrix of the error,

H= sum squares matrix of the hypothesis ,

VE = degrees of freedom for error,

VH = degrees of freedom for hypothesis. We reject hypothesis If  $\frac{VE}{VH} U^{(s)} >$  table value.

The One-way MRM ANOVA are given in the following table(1) .

	Source	D.F	SS	Lawley-Hotelling Criterion
Between	Group	q-1	$SS_G$	trace $(SS_G SS_{u(G)}^{-1})$
	Unit(Group)	n-q	$SS_{u(G)}$	
Within	Time	(p-1)	$SS_T$	trace $(SS_T SS_E^{-1})$
	Group $\times$ Time	(q-1)(p-1)	$SS_{G \times T}$	
	Error	(p-1)(n-q)	SSE	trace $(SS_{G \times T} SS_E^{-1})$

## 6 – The Experiment

The data of the experiment was taken from Date palm research center, Basrah university – which represent for isolation and identification of bacterial types that contaminated date palm tissue culture., and studied the inhibiting activities of three types of plant extracts on fruit of *Rhus coriaria* , bark of *Cinnamomum zeylanicum* and gummy extraction of *Bswellia* sp., using four types of solvent water , methyl alcohol , normal hexane and ethyl acetate , in two concentrations (0.5 , 1) % . The results of isolation and identification of bacteria appeared contamination of callus tissue of date palm tissue culture by three genera of bacteria *Staphylococcus aurwus* , *Bacillus subtilus* and *proteus* spp .

## 7- The results and discussion

According to the mathematical formula of the model study (2.1) and by applying the model to the experiment, we get the sum squares matrices , of the effects between-units factors, within-units factors, effect to interaction between-unit factor (group ) and within-units factors(time), of the experiment as table(2) We reject hypotheses  $H_{01}$ ,  $H_{02}$  and  $H_{03}$  ,that by using Lawly-Hotelling test at 0.05 level of significance, because of the calculated Lawly-Hotelling greater then table values. In rejection our hypothesis  $H_{01}$  ,we mean that the group factor has active effect, and each one of *Rhus coriaria* , *Cinnamomum zeylanicum* , *Bswellia* sp has different affection on frustrate core of bacteria. In point of hypothesis  $H_{02}$ , mean our rejection that for each one of bacteria kinds (time factor) will be touching effectually.  $H_{03}$  is rejected because we can find affection for the interaction between group factor and time factor, and we can say that, the results showed that ethyl acetate extracts 1% of each *Rhus coriaria* and *Bswellia* sp. appeared the highest inhibition zone of about (23.00 , 24.00)mm respectively against *S.aurwus* while the inhibition zone of ethyl acetate acetate extract 1% of *Bswellia* sp.against *B.subtillus* was 18.33 mm , the results showed that alcohol and ethyl for



acetate Rhus coriaria extract were the best extracts that gave the highest inhibition zone (16.76,16.33) mm respectively against Proteus sp.

Table(2)

	hypotheses	Calculated value of Lawly-Hotelling	Table value of Lawly-Hotelling	The comparison	The decision
<b>Between</b>	$H_{01}: \tau_1^* = \tau_2^* = \tau_3^* = 0$	31.32	7.91	$31.32 > 7.91$	Rejection $H_{01}$
<b>Within</b>	$H_{02}: \gamma_2^* = \gamma_3^* = 0$	22.95	6.19	$22.95 > 6.19$	Rejection $H_{02}$
<b>Interacti-on between and within</b>	$H_{03}: (\tau\gamma)_{j2}^* = (\tau\gamma)_{j3}^* = 0$	112.5	5.21	$112.5 > 5.21$	Rejection $H_{03}$

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