TESTING FOUR TREATMENTS USING 6 X 6 LATIN SQUARE

N a s r u l l a h *

R I N G K A S A N

Karangan ini mengetengahkan cara analisa statistik percobaan yang menguji empat perlakuan dengan rancangan lingkungan Rancangan Segi Empat Latin 6 x 6 yang setimbang baik pada baris maupun kolom. Rancangan ini dipakai dalam usaha memperoleh tambahan derajad bebas untuk mendapatkan nilai duga varians kesalahan percobaan yang memadai yang tidak mungkin dicapai dengan menduakalikan Rancangan Segi Empat Latin 4 x 4 tanpa mengorbankan pertimbangan lain yaitu ukuran terkecil petak yang diperlukan sehubungan dengan terbatasnya unit percobaan yang tersedia.

Suatu contoh numeris pemakaiannya juga diberikan.

S U M M A R Y

Statistical procedure for analyzing experiment testing four treatments in 6 x 6 Latin Square which is balanced in both row and column was described. The design is selected to get sufficient degrees of freedom to give an appropiate estimate of experimental error, since it is not possible to duplicate the 4 x 4 Latin Square without sacrifying another consideration, the minimum plot size, because of the limited experimental material.

A numerical example was given for clarification of the procedure.

I N T R O D U C T I O N

Trail and Weeks (1973) described a general statistical method for analyzing experiments arranged in an extended complete block design generated by balanced incomplete block design, a design in which some, but not all, treatments are duplicated within each block.

In field experiments, it is frequently happened that soil fertility and other variations in two directions are present. Likewise, in greenhouse experiments, there is variation along and across the greenhouse bench. If this is the case, Latin Square is an apllicable skillful design.

Restriction of using Latin Square is on the requirement of having rows, columns, and treatments equally numerous to keep the design simple. Thus, to test four treatments, the square must be divided into four rows and four columns. The treatments are assigned so that each occurs once and only once in each row and each column. Due to few degrees of freedom available to give an appropiate estimate of experimental error, it is advised to double the 4 x 4 Latin Square (Kempthorne, 1960; Steel & Torrey, 1960). Sometimes it is happened that the square is somewhat large, in the sense that the division into rows and columns gives plot size larger than that required, but it is not possible to double the 4 x 4 Latin Square without sacrifying another consideration, namely the minimum plot size. The maximum number of rows and of columns the square can accomodates, taking the requirement of plot size into account is six. One method to solve the problem is introducing new treatments to make the number of treatments equals to the number of rows and of columns. But it is frequently not intended, as the new treatments are out of interest. The other method is using a balanced three way classification in which each row and each column contains one complete replicate of the treatments and a certain number of additional treatments, which are balanced in both rows and columns (Pearce, 1953).

Considering a statistical method for such experiments is the objective of the paper.

STATISTICAL ANALYSES

An example lay out to test four treatments using 6 x 6 Latin Square is illustrated in Fig. 1 (Pearce, 1953).

B	A	C	D	B	A
D	A	B	A	C	C
A	C	D	B	C	D
C	B	A	A	D	D
C	C	B	B	D	A
B	D	D	C	A	B

Fig. 1

In the model given below, ρ_i, γ_j, and τ_k are used to represent the effect of i^{th} row, j^{th} column, and k^{th} treatment respectively. Furthermore, it is assumed that there are no interaction among the three factors. Y_{ijk} is the yield of row i, column j which recieves treatment k. Hence

$$Y = mj_1^{36} + X_1\rho + X_2\gamma + X_3\tau + e \qquad (1)$$

is the matrix model, where

$$Y = \begin{bmatrix} Y_{112} \\ Y_{121} \\ . \\ . \\ Y_{662} \end{bmatrix} \quad X_1 = \begin{bmatrix} j_1^6 & & & \\ & j_1^6 & & \\ & & . & \\ & & & j_1^6 \end{bmatrix} \quad \rho = \begin{bmatrix} \rho_1 \\ \rho_2 \\ . \\ . \\ \rho_6 \end{bmatrix} \quad X_2 = \begin{bmatrix} I_6 \\ I_6 \\ . \\ . \\ I_6 \end{bmatrix}$$

$$\gamma = \begin{bmatrix} \gamma_1 \\ \gamma_2 \\ . \\ . \\ \gamma_6 \end{bmatrix} \quad X_3 = \begin{bmatrix} 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ . & . & . & . \\ . & . & . & . \\ 0 & 1 & 0 & 0 \end{bmatrix} \quad \tau = \begin{bmatrix} \tau_1 \\ \tau_2 \\ . \\ . \\ \tau_4 \end{bmatrix} \quad e = \begin{bmatrix} e_{112} \\ e_{121} \\ . \\ . \\ e_{662} \end{bmatrix}$$

The constants associated with the design are : number of rows, number of columns, number of treatments, number of application of each treatment, number of distinct pairs of experimental units which recieve any fixed pair of treatments while appearing in the same row and that in the same column. They are denoted by r, c, t, s, λ_r and λ_c which are equal to 6, 6, 4, 9, 13, and 13 respectively.

The error sum of squares is

$$e'e = Y^{*\prime}Y^{*} - 2Y^{*\prime}X_1\rho - 2Y^{*\prime}X_2\gamma - 2Y^{*\prime}X_3\tau + \rho' X_1' X_1 \rho + \gamma' X_2' X_2 \gamma + \tau' X_3' X_3 \tau + 2\rho' X_1' X_2 \gamma + 2\rho' X_1' X_3 \tau + 2\gamma' X_2' X_3 \tau \quad (2)$$

where

$$Y^{*} = Y - mj_1^{36} \quad (3)$$

Differentiating (2) with respect to ρ, γ, and τ alternatively, and each is equated to zero, we get after dividing by 2

$$X_1' Y^{*} = X_1' X_1 \rho + X_1' X_2 \gamma + X_1' X_3 \tau \quad (4)$$

$$X_2' Y^{*} = X_2' X_1 \rho + X_2' X_2 \gamma + X_2' X_3 \tau \quad (5)$$

$$X_3' Y^{*} = X_3' X_1 \rho + X_3' X_2 \gamma + X_3' X_3 \tau \quad (6)$$

Let $\hat{\rho}$, $\hat{\gamma}$, and $\hat{\tau}$ is the estimate of ρ, γ, and τ. By the use of Gauss sweep out method, the normal equations under restrictions of $j'\hat{\rho} = 0$, $j'\hat{\gamma} = 0$, and $j'\hat{\tau} = 0$ become

$$\hat{\rho} = (X_1' X_1)^{-1} (X_1' Y^{*} - X_3' \hat{\tau}) \quad (7)$$

$$\hat{\gamma} = (X_2' X_2)^{-1} (X_2' Y^{*} - X_3' \hat{\tau}) \quad (8)$$

$$\hat{\tau} = [X_3' X_3 - X_3' X_1 (X_1' X_1)^{-1} X_1' X_3 - X_3' X_2 (X_2' X_2)^{-1} X_2' X_3]^{-1} [X_3' Y^{*} - X_3' X_1 (X_1' X_1)^{-1} X_1' Y^{*} - X_3' X_2 (X_2' X_2)^{-1} X_2' Y^{*}] \quad (9)$$

Substituting (4), (5), and (6) to (2) gives

$$e'e = Y^{*\prime}Y^{*} - Y^{*\prime}X_1\hat{\rho} - Y^{*\prime}X_2\hat{\gamma} - Y^{*\prime}X_3\hat{\tau} \quad (10)$$

and using (7) and (8), (10) becomes

$$e'e = Y^{*\prime}Y^{*} - Y^{*\prime}X_1(X_1' X_1)^{-1} X_1' Y^{*} - Y^{*\prime}X_2(X_2' X_2)^{-1} X_2' Y^{*} - [Y^{*\prime}X_3 - Y^{*\prime}X_1(X_1' X_1)^{-1} X_1' X_3 - Y^{*\prime}X_2(X_2' X_2)^{-1} X_2' X_3]\hat{\tau}$$

Finally, inserting (9) we get

$$e'e = Y^{*\prime}Y^{*} - Y^{*\prime}X_1(X_1' X_1)^{-1} X_1' Y^{*} - Y^{*\prime}X_2(X_2' X_2)^{-1} X_2' Y^{*} - [X_3' Y^{*} - X_3' X_1(X_1' X_1)^{-1} X_1' Y^{*} - X_3' X_2(X_2' X_2)^{-1} X_2' Y^{*}]' [X_3' X_3 - X_3' X_1(X_1' X_1)^{-1} X_1' X_3 - X_3' X_2(X_2' X_2)^{-1} X_2' X_3]^{-1} [X_3' Y^{*} - X_3' X_1(X_1' X_1)^{-1} X_1' Y^{*} - X_3' X_2(X_2' X_2)^{-1} X_2' Y^{*}] \quad (11)$$

Denoting $A = I - X_1(X_1' X_1)^{-1} X_1' - X_2(X_2' X_2)^{-1} X_2'$, the analysis of variance is shown in Table 1.

Table 1. Analysis of Variance

Source of Variation	df	Sum of Square	E (MS)
Row (unadj.)	$r - 1$	$Y^{*\prime} X_1(X_1' X_1)^{-1} X_1' Y^*$	
Column (unadj.)	$r - 1$	$Y^{*\prime} X_2(X_2' X_2)^{-1} X_2' Y^*$	
Treatment (adj.)	$t - 1$	$Y^{*\prime} AX_3(X_3' AX_3)^{-1} X_3'AY^*$	$\sigma_e^2 + \frac{2\lambda t - sr}{r(t-1)} \sum(\tau - \bar{\tau})^2$
Error	$(r-1)^2 - (t-1)$	By substraction	σ_e^2
Total	$r^2 - 1$	$Y^{*\prime} Y^*$	

ESTIMATION OF TREATMENT EFFECTS

Recall equation (9), $C\hat{\tau} = X_3'Y^* - X_3'X_1(X_1'X_1)^{-1}X_1'Y^* - X_3'X_2(X_2'X_2)^{-1}X_2'Y^*$ where $C = X_3'X_3 - X_3'X_1(X_1'X_1)^{-1}X_1'X_2 - X_3'X_2(X_2'X_2)^{-1}X_2'X_3$. As consequence of the earlier definitions, it may be seen that

$X_1'X_1 = rI$

$X_2'X_2 = cI$

$X_3'X_3 = tI$

$X_1'X_2 = J$

$X_1'X_3 = N$ (incidence matrix ignoring the column)

$X_2'X_3 = M$ (incidence matrix ignoring the row)

$r = c$

$rc = st$

$\lambda_c = \lambda_r = \lambda$

$N'N = J + (rs - t)I$

$M'M = J + (cs - t)I$

$N'N = M'M$

So

$$C = \frac{2\lambda t - sr}{r}\left[I - \frac{2}{2\lambda t - sr} J\right]$$

and the variance of the difference of the two treatment means is

$$Var(\hat{\tau}_k - \hat{\tau}_{k'}) = \frac{2r}{2\lambda t - sr}\sigma_e^2$$

NUMERICAL EXAMPLE

The table below is a hypothetical yield of experiment whose lay out is shown in Fig. 1.

B = 55.8	A = 28.0	C = 45.3	D = 47.2	B = 52.4	A = 29.6
D = 47.2	A = 28.8	B = 48.5	A = 34.8	C = 47.4	C = 48.2
A = 33.7	C = 43.5	D = 46.3	B = 48.4	C = 39.1	D = 45.5
C = 41.0	B = 40.8	A = 41.7	A = 42.0	D = 41.7	D = 42.5
C = 38.5	C = 38.2	B = 36.7	B = 35.2	D = 41.6	A = 37.0
B = 41.2	D = 33.9	D = 35.3	C = 36.2	A = 45.2	B = 39.4

Total of rows (R_i), total of column (C_j), total of treatment (T_k), and general total are

$R_1 = 258.3$	$C_1 = 257.4$	$T_1 = 320.8$	GT = 1,477.8
$R_2 = 254.9$	$C_2 = 213.2$	$T_2 = 398.4$	
$R_3 = 256.5$	$C_3 = 253.8$	$T_3 = 377.4$	
$R_4 = 249.7$	$C_4 = 243.8$	$T_4 = 381.4$	
$R_5 = 227.2$	$C_5 = 267.4$		
$R_6 = 231.2$	$C_6 = 242.2$		

$$m = \frac{1,477.8}{36} = 41.05$$

$$CF = \frac{(1,477.8)^2}{36} = 60,663.69$$

Total Sum of Squares

$$= (55.8)^2 + (47.2)^2 + \ldots + (39.4)^2 - CF$$
$$= 62,122.54 - 60,663.69$$
$$= 1,458.85$$

$$R_{SS} = \frac{(258.3)^2 + (254.9)^2 + \ldots + (231.2)^2}{6} - CF$$
$$= 60,818.09 - 60,663.69$$
$$= 154.40$$

$$C_{SS} = \frac{(257.4)^2 + (213.2)^2 + \ldots + (242.2)^2}{6} - CF$$
$$= 60,954.25 - 60,663.69$$
$$= 290.56$$

$X'_3 Y^* - X'_3 X_1(X'_1 X_1)^{-1} X'_1 Y^* - X'_3 X_2(X'_2 X_2)^{-1} X'_2 Y^*$ is

$$\begin{bmatrix} 320.8 \\ 398.4 \\ 377.4 \\ 381.2 \end{bmatrix} - \frac{1}{6}\begin{bmatrix} 2 & 2 & 1 & 2 & 1 & 1 \\ 2 & 1 & 1 & 1 & 2 & 2 \\ 1 & 2 & 2 & 1 & 2 & 1 \\ 1 & 1 & 2 & 2 & 1 & 2 \end{bmatrix}\begin{bmatrix} 258.3 \\ 254.9 \\ 256.5 \\ 249.7 \\ 227.2 \\ 231.2 \end{bmatrix} - \frac{1}{6}\begin{bmatrix} 1 & 2 & 1 & 2 & 1 & 2 \\ 2 & 1 & 2 & 2 & 1 & 1 \\ 2 & 2 & 1 & 1 & 2 & 1 \\ 1 & 1 & 2 & 1 & 2 & 2 \end{bmatrix}\begin{bmatrix} 257.4 \\ 213.2 \\ 253.8 \\ 243.8 \\ 267.4 \\ 242.2 \end{bmatrix} + \begin{bmatrix} 369.45 \\ 369.45 \\ 369.45 \\ 369.45 \end{bmatrix}$$

and equal to $\begin{pmatrix} -46.03 \\ 29.97 \\ 8.15 \\ 7.92 \end{pmatrix}$

$$T_{SS} = \frac{6}{50} \begin{pmatrix} -46.03 \\ 29.97 \\ 8.15 \\ 7.92 \end{pmatrix}' \begin{pmatrix} -46.03 \\ 29.97 \\ 8.15 \\ 7.92 \end{pmatrix} = 377.54$$

$$E_{SS} = 1,458.85 - 154.40 - 290.56 - 377.54$$
$$= 636.35$$

The Analysis of Variance is shown below

Analysis of Variance

Source of Var.	df	SS	MS	F
Row (unadj.)	5	154.40		
Column (unadj.)	5	290.56		
Treatment (adj.)	3	377.54	125.85	4.35**
Error	22	636.35	28.93	
Total	35	1,458.85		

** Significant at 1% level of significance

Estimation of the treatment effects is shown below

$$\hat{\gamma} = \frac{6}{50} \begin{bmatrix} -46.03 \\ 29.97 \\ 8.15 \\ 7.92 \end{bmatrix} = \begin{bmatrix} -5.524 \\ 3.596 \\ .978 \\ .950 \end{bmatrix}$$

with variance of two treatments difference

$$\text{Var } (\hat{\gamma}_k - \hat{\gamma}_{k'}) = \frac{12}{50} (28.93)$$
$$= 6.94$$

ACKNOWLEDGEMENT

The author is indebted to Ir. Soemartono, Faculty of Agriculture, Gadjah Mada University, for his invaluable help and encouragement, to Prof. M.M. Grossman from University of Illinois for his suggestion given when he visited the Faculty of Animal Husbandry, Gadjah Mada University, in 1974, to include a numerical example, and to Dr. Ir. Setiono, Faculty of Forestry, Gadjah Mada University, for his advice. For helpful discussion on writing, the author wish to thank to Ir. C. Winarko.

REFERENCES

Kempthorne, O. (1960) The Design And Analysis of Experiments. John Wiley & Sons, New York.

Pearce, S.C. (1953) Field Experimentation with Fruit Trees And Other Perennial Plants. Commonwealth Bureau of Horticulture And Plantation Crops, East Malling.

Steel, R.D. and J.H. Torrie (1960) Principles and Procedures of Statistics : with emphasis on agriculture. McGraw-Hill, New York.

Trail, S.M. and D.L. Weeks (1973) Extended Complete Block Designs Generated by BIBD. *Biometrics* 29 : 565 – 578.

Catatan untuk pengarang.

Naskah dapat ditulis dalam bahasa Indonesia atau bahasa Inggris, diketik pada satu muka, dengan kertas putih ukuran kwarto, dengan jarak 1½ spasi, kecuali : Ringkasan, Summary, Tabel, Keterangan gambar, Daftar pustaka, dan keterangan lain, diketik dengan 1 spasi.

Naskah harus dalam ketikan asli, bukan tembusan, harus dibuat yang jelas dan rajin dengan diberi ruang tepi (margin) yang cukup. Tembusan naskah disimpan pengarang.

Gambar, grafik, lukisan-lukisan lain, digambar dengan tinta CINA, paling besar sama dengan ukuran majallah ini (kwarto).

Gambar potret, jika ada, harus pada kertas mengkilat, jelas dan tidak kabur.

Untuk menghemat mahalnya ongkos penerbitan, harap jumlah potret dibatasi melulu untuk hal-hal yang perlu sekali.

Gambar-gambar (lukisan atau potret) dan tabel diberi nomer urut dalam naskah itu dan dan letaknya gambar perlu diberi petunjuk pada tepi (margin) kertas tersebut. Tabel, gambar, potret, diberi judul, keterangan singkat, satuan ukuran, dan nomer urut.

Nama Latin (binomial) diberi garis bawah.

Jika tidak memakai mesin tik khusus, maka rumus-rumus dan persamaan ilmu pasti, dapat ditulis tangan asal jelas.

Sedapat mungkin naskah disusun sebagai berikut :

1. Judul, disertai dengan terjemahannya dalam bahasa Inggris.
2. Nama pengarang, keterangan tempat bekerja dalam "footnotes".
3. Summary. (Dalam bahasa Inggris).
4. Ringkasan.
5. Pengantar (Introduction), kalau perlu dapat ditambah Ikhtisar pustaka, keterangan singkat mengenai situasi (alam, kebudayaan dan lain-lain) tempat percobaan.
6. Bahan dan cara .(Materials and methods).
7. Hasil (Results).
8. Pembahasan (Discussion).
9. Kesimpulan (Conclusion).
   Pembahasan (Discussion) dan Kesimpuan (Conclusion) dapat ditulis dalam satu bab. Demikian juga Hasil (Results) dan Pembahasan (Discussion).
10. Ucapan terima kasih (Acknowledgement), bila perlu.
11. Daftar pustaka :
    a. Nama pengarang, disusun menurut abjad.
    b. Tahun penerbitan ditulis dalam kurung.
       Jika karangan berikutnya diterbitkan oleh pengarang dalam tahun yang sama, maka pada tahun penerbitan ditambahkan huruf-huruf a, b, c, dan seterusnya.
    c. Judul lengkap dari karangan.
    d. Nama majallah dapat disingkat dalam bentuk yang umum digunakan.
    e. Jilid (Volume).
    f. Halaman pertama dan akhir dari karangan.
       Bahan referensi dari buku sebaiknya meliputi nama pengarang (disusun menurut abjad), tahun penerbitan (dalam kurung), judul buku, edisi, penerbit dengan tempat penerbitan dan jumlah halaman.

Footnotes tidak digunakan kecuali untuk personal communication".

Pengarang harap membatasi karangannya antara 10—30 halaman kwarto.

Redaksi mempunyai hak untuk menyusun naskah sedemikian sehingga sesuai dengan cara-cara yang dikehendaki atau mengembalikannya untuk diperbaiki, atau menolak naskah yang bersangkutan.

Hanya naskah-naskah yang disertai perangko secukupnya, dengan amplop, akan dikirimkan kembali bila diminta.

Naskah-naskah yang sudah diterbitkan dalam majallah lain dapat diterima bila dipandang perlu untuk diterbitkan lagi. Nama majallah yang telah menerbitkan karangan tersebut perlu di terangkan dalam naskah.

Pengarang akan menerima cuma-cuma 25 ex (reprint) untuk karangannya yang dimuat dalam majallah ini.

Notice to Contributors.

Manuscripts can be written in Indonesian or English, and should be submitted type-written one and a half spaced on one side only of good white paper approximately 8½ X 11 inches, except for summaries, tables, text of illustrations, legends, list of references and other subsidiary, which should be typed single spaced.

Only the original copy of the manuscript is required. It should be neatly and clearly prepared, leaving a sufficient margin all around. One carbon copy must be retained by the author

Line drawings should be made neatly with black Indian ink on heavy white drawing paper. Photographs, if any, should have a glossy finish, be unblurred, and show sharp contrast between light and dark areas. To avoid unnecessarily high cost of printing, the number of photographs should be limited to the most indispensable ones.

Illustrations (line drawings or photographs) and tables should be numbered consecutively throughout the manuscript and approximate place of insertion should be indicated in margins of text paper. Tables, line drawings, and photographs should be completed with the appropriated headings, legends, short explanatory text, unit of measurements, and consecutive numbers.

Biological Latin names should be underlined.

If a mathematical type writer is not available, all mathematical symbols in equations and formulas should be clearly written by hand.

Manuscripts should be divided into the following parts in the following arrangement :

1. Title
2. Name of author (s) and affiliation.
   Senior author first. Use footnotes for author (s) affiliation.
3. Summary.
4. Introduction.
   Literature riview and general situation of the site (Natural, cultural and administrative, for articles on field experiments, soil surveys etc) if needed.
5. Materials and methods.
6. Results.
7. Discussion.
8. Conclusions.
   For simplicity, results and disccussion or discussion and conclusion may be combined.
9. Acknowledgement.
10. References :
    a. Arranged alphabetically by author.
    b. Year of publication between brackets; if more than one article appeared in the same year by the same author (s) use additional small letters a, b, c, etc.
    c. Full title of article.
    d. Name of periodicals abbreviated according to List of Periodicals Abstracted in current usage; if in doubt give name in full.
    e. Volume number.
    f. First and last page of article.

For books the following order of stating applies : name of author (s), year of edition between brackets, full title, edition, name and place of publisher, and number of pages (included which are numbered with Roman numbers).

Do not cite references in footnotes, except for "personal communications".

Each manuscript should be no longer than about 30 pages, including tables, illustrations, and list of references.

The editor preserve the rights to rearrange any submitted manuscript as to conform it with the adopted style of this journal, or for returning it to the contributor (s) for revision, or of not accepting manuscripts.

Author(s) of not accepted manuscripts will be given notice on the matter. The manuscript will be returned only on request and if has been provided with a sufficiently stamped envelope.

Articles which were already published in another journal could also be accepted if considered important enough to be published again. The name of the journal in which it has been previously published should be stated in the manuscript.

Contributors will receive 25 separates of each accepted article free of charge.