Year	Subject Title	Sem	Sub Code
2018–19 Onwards	**Core VIII: DESIGN OF EXPERIMENTS**	**III**	**18MST32C**

Objective: To impart the knowledge and applications of various advanced Design of Experiments in the field of Agriculture and Industries.

UNIT –I

Basic Principles of Experimentation – Experimental Error – Review of CRD & RBD - LSD – Applications – Layout of LSD – Advantages and Disadvantages of LSD – Statistical Analysis of LSD – Least Square Estimates of parameters – Multiple comparison methods – Least Significant Difference method – DMRT and Tukey's Test.

UNIT –II

Factorial experiments – Introduction – 2^2 factorial Design – Statistical Analysis of 2^2 factorial Design – Yates method of computing 2^2 factorial totals. 2^3 Factorial Experiment – Model Description - Statistical Analysis of 2^3 factorial Design- Yates method of computing 2^3 factorial totals – 3^2 Factorial Experiment - Confounding – Partial confounding and complete Confounding.

UNIT – III

Split Plot Design – Introduction – model description – Statistical Analysis – Advantages and Disadvantages. - Analysis of Covariance with one Concomitant variable – model – Least Square Estimates for parameters – Estimation of variance – Statistical analysis in CRD & RBD.

UNIT –IV

Incomplete Block Designs – Introduction – Balanced Incomplete Block Designs – Parametric Relationships – Symmetric BIBD – Statistical Analysis of Balanced Incomplete Block Designs (Intra Block only) - Partial BIBD.

UNIT –V

Response surface methodology – Introduction – First Order Design – Model – Statistical Analysis of Response surface first order design – Design for Bio-assays – Direct, Indirect and Parallel line assays.

Text Books:

1. S.C. Gupta and V.K. Kapoor : Fundamental of Applied Statistics – Sultan Chand & Sons, Fourth Edition, 2015.
2. R. Panneer Selvam : Design And Analysis of Experiments, Prentice Hall.
3. M.N. Das and N.P. Giri : Design and Analysis of Experiments, New Age International, 2nd Edtion, 2008.

Reference Books:

1. W.G. Cochran and G.M. Cox : Experimental Designs – John Wiley.
2. Montgomery : Design and Analysis of Experiments, John Wiley & Sons (p) Ltd.. 5th Edition 2009.
3. Angela Dean and Daniel Voss : Design and Analysis of Experiments, Springer.

Year	Subject Title	Sem	Sub Code

6·1. INTRODUCTION

In 1935 **Sir Ronald A. Fisher** laid the foundation for the subject which has come to be known by the title of his book '*The Design of Experiments*'. Since then the theory of experimental design has been considerably developed and extended. Applications of this theory are found today in laboratories and research in natural sciences, engineering, and nearly all branches of social science.

Experimentation provides what is called experimental data in contrast to observational data with which we have been mainly concerned up to this point. *Observational data* are represented by observations on the elementary units of a population or of a sample and are not changed or modified by any attempt on the part of an investigator during the course of observation. It is often difficult to assign cause and effect by studying observational data. If one is interested in establishing causal relationships, he should work with *experimental data*, data arising from observations on a universe or a segment thereof which have been 'controlled' or modified by varying certain factors in order to determine what effect, if any, the factors have on the data. In other words, experimental data are the results from logically designed experiments which provide evidence for or against theories of cause and effect.

The subject-matter of the design of experiment includes :

(*i*) Planning of the experiment,

(*ii*) Obtaining relevant information from it regarding the statistical hypothesis under study, and

(*iii*) Making a statistical analysis of the data.

Experience has shown that proper consideration of the statistical analysis before the experiment is conducted, forces the experimenter to plan more carefully the design of the experiment. The observations obtained from a carefully planned and well-designed experiment in advance give entirely valid inferences. However, the certainty of the conclusion so drawn, regarding the acceptance or rejection of the null hypothesis, is given only in terms of probability. Accordingly, the *Design of Experiment* may be defined as "*the logical construction of the experiment in which the degree of uncertainty with which the inference is drawn may be well defined.*"

In the words of Allen L. Edwards, "*The experimental design is called a randomised group design. The essential characteristic of this design is that subjects are randomly assigned to the experimental treatments or vice versa.*

The experimenter may easily recognise three important phases of every project :

I. Experimental or Planning Phase,

(*i*) Statement of problem.

(*ii*) Choice of response or dependent variable.

(*iii*) Selection of factors to be varied.

(*iv*) Choice of levels of these factors.

(*a*) Quantitative or qualitative.

(*b*) Fixed or random.

(*v*) How factor levels are to be combined ?

II. Design Phase

(*i*) Number of observations to be taken.

(*ii*) Order of experimentation.

(*iii*) Method of randomisation to be used.
(*iv*) Mathematical model to describe the experiment.
(*v*) Hypothesis to be tested.

III. Analysis Phase

(*i*) Data collection and processing.
(*ii*) Computation of test statistics.
(*iii*) Interpretation of results for the experimenter.

Remark. It may be noted that experimental design is a very broad and intensively investigated field. Entire books have been written on only limited phases of this subject. In this chapter we shall confine ourselves to the study of some of the simple but highly useful types of experimental designs frequently employed in business, economic and scientific researches.

The reader may be warned before he proceeds further that this chapter has the appearance of the most difficult one in this text up to now. The difficulty arises mainly from using rather involved summation signs and notation, and from the rather heavy computational labour even with the simple examples for various models introduced. To avoid confusion from the first source, the reader is advised to study with great care each of the definitions of various sums and statistics. He/She must be perfectly sure about each mathematical expression as he/she proceeds step by step. As to the second source of difficulty, problems involving the analysis of variance are usually solved with computer programmes. However, it may be noted, computers are no substitute for underlying principles. It is, therefore, necessary, although somewhat painful, for the reader to go through this chapter thoroughly before he/she can take advantage of computers as a computational aid.

6·2. TERMINOLOGY IN EXPERIMENTAL DESIGNS (Important Terms and Definitions)

A number of basic aspects used in the context of the theory of experimental design are worth noting at the very beginning.

Experiment. An experiment is a device or a means of getting an answer to the problem under consideration. Experiment can be classified into two categories : (*a*) Absolute, and (*b*) Comparative.

Absolute experiments consist in determining the absolute value of some characteristics like (*i*) obtaining the average intelligence quotient (I.Q.) of a group of people, (*ii*) finding the correlation coefficient between two variables in a bivariate distribution, etc. On the other hand, *comparative experiments* are designed to compare the effect of two or more objects on some population characteristic, *e.g.,* comparison of different *manures* or fertilizers, different kinds of varieties of a crop, different cultivation processes, different pieces of land in a field experiment, or different diets or medicines in a dietary or medical experiment respectively.

Treatments. Various objects of comparison in a comparative experiment are termed as treatments, *e.g.,* in field experimentation different fertilizers or different varieties of crop or different methods of cultivation are the treatments.

First of all, many experiments are conducted to establish the effect of one or more (independent) variables on a response (the dependent variable). Here, the independent variables are often called *treatments* or *factors,* which are often qualitative in nature, such as different makes of machines, different advertisement channels, different ways of packaging merchandise, and so on. The values of a *response* are supposed to reflect the effects of different treatments.

Experimental Unit. The smallest division of the experimental material to which we apply the treatments and on which we make observations on the variable under study, is termed as experimental unit, *e.g.,* in field experiments the plot of 'land' is the experimental

unit. In other experiments, unit may be a patient in a hospital, a lump of dough or a batch of seeds.

Blocks. In agricultural experiments, most of the times we divide the whole experimental unit (field) into relatively homogeneous sub-groups or strata. These strata, which are more uniform amongst themselves than the field as a whole, are known as *blocks.*

Yield. The measurement of the variable under study on different experimental units (*e.g.*, plots, in field experiments) are termed as *yields.*

Experimental Error. Let us suppose that a large homogeneous field is divided into different plots (of equal shape and size) and different treatments are applied to these plots. If the yields from some of the treatments are more than those of the others, the experimenter is faced with the problem of deciding if the observed differences are really due to treatment effects or they are due to chance (uncontrolled) factors. In field experimentation, it is a common experience that the fertility gradient of the soil does not follow any systematic pattern but behaves in an erratic fashion. Experience tells us that even if the same treatment is used on all the plots, the yields would still vary due to the differences in soil fertility. Such variation from plot to plot, which is due to random (or chance or non-assignable) factors beyond human control, is spoken of as *experimental error.* It may be pointed out that the term '*error*' used here is not synonymous with 'mistake' but is a technical term which includes all types of extraneous variations due to :

(*i*) the inherent variability in the experimental material to which treatments are applied,

(*ii*) the lack of uniformity in the methodology of conducting the experiment or in other words failure to standardise the experimental technique, and

(*iii*) lack of representativeness of the sample to the population under study.

Remark. In order to test the significance of the difference between two treatments, we first obtain an estimate of the experimental error and then apply some test of significance. The former is achieved through *replication* and for the latter we allocate the treatments to various plots at random. It is also desired to keep the experimental error as small as possible so that even smaller real differences can be detected. The experimental error is controlled through the principle of '*local control*'. The terms replication, randomisation and local control are discussed in detail in next section (§ 6·3).

Replication. Replication mans the execution of an experiment more than once. In other words, the repetition of treatments under investigation is known as replication.

Precision. The reciprocal of the variance of the mean is termed as the *precision, or the amount of information* of a design. Thus for an experiment replicated r times, the precision is given by :

$$\frac{1}{\text{Var}(\bar{x})} = \frac{r}{\sigma^2} \qquad \ldots (6\cdot1)$$

where σ^2 is the error variance per unit.

Efficiency of a Design. Consider the designs D_1 and D_2 with error variances per unit σ_1^2 and σ_2^2, and replications r_1 and r_2 respectively. Then the variance of the difference between two treatment means is given by :

$2\sigma_1^2/r_1$ and $2\sigma_2^2/r_2$ for D_1 and D_2 respectively. Then the ratio

$$E = \frac{2\sigma_2^2}{r_2} \times \frac{r_1}{2\sigma_1^2} = \frac{r_1}{\sigma_1^2} + \frac{r_2}{\sigma_2^2} \qquad \ldots (6\cdot2)$$

is termed as efficiency of design D_1 *w.r.t* D_2. In other worlds, efficiency of D_1 *w.r.t.* D_2 may be defined as the '*ratio of the precisions of* D_1 and D_2'.

If $E = 1$, then both the designs D_1 and D_2 are said to be equally efficient.

If $E > 1$ ($E < 1$), then D_1 is said to be more (less) efficient than D_2.

Remark. An efficient or sensitive design has greater ability of detecting the differences of treatment effects. It is obvious from (6·1) that the efficiency of a design can be increased by

(*i*) controlling, *i.e.*, decreasing σ^2, the error variance per unit. This is done by arranging the plots into small homogeneous blocks, and

(*ii*) increasing r, the number of replications.

Uniformity Trials. As has been pointed out earlier, the fertility of the soil does not increase or decrease uniformly in any direction but is distributed over the entire field in an erratic manner. *Uniformity trials* enable us to have an idea about the fertility variation of the field. By uniformity trial, we mean a trial in which the field (experimental material) is divided into small units (plots) and the *same* treatment is applied on each of the units and their yields are recorded. From these yields, we can draw a '*fertility contour map*' which gives us a graphic picture of the variation of the soil fertility and enables us to form a good idea about the nature of the soil fertility variation. The fertility contour map is obtained by joining the points of equal fertility through lines.

Accordingly the field (which is expected to be heterogeneous *w.r.t.* fertility) can be divided into relatively homogeneous sub-groups (blocks) to control the experimental error. Incidently, uniformity trials also give as some idea about the shape and size of the plots to be used.

Remark. From the fertility contour map, it is generally observed that adjacent plots are more or less alike in fertility than those apart. Thus a homogeneous block can be formed by combining a number of adjacent plots.

6·3. THREE PRINCIPLES OF EXPERIMENTAL DESIGN

For the validity of statistical analysis and enhancing the precision of the experiments, three basic principles : (*a*) replication, (*b*) randomisation, and (*c*) local control are observed according to Prof. **Ronald A. Fisher** who pioneered the study of experimental design in his classical book, '*The Design of Experiments*'. The *Fig. 6·1.* due to Fisher illustrates the functions of the various principles.

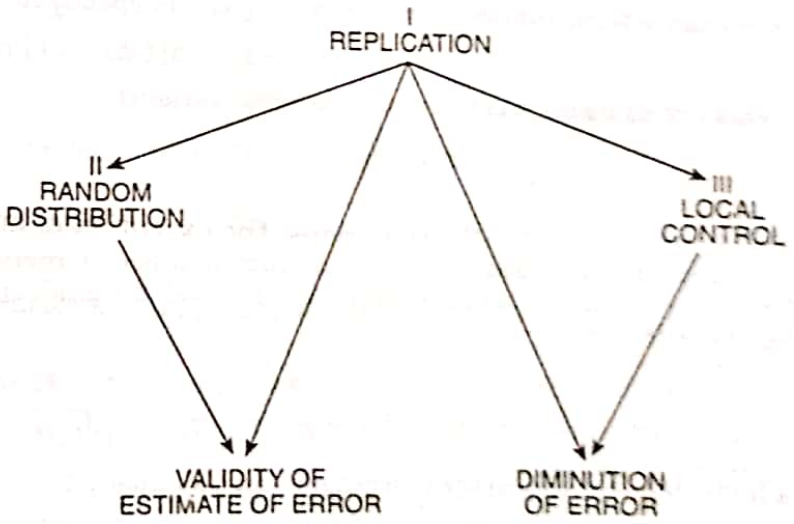


Fig. 6·1 : Fisher's Diagram

The design of an experiment may assume a relatively simple form where only one treatment variable is considered or a quite complicated framework involving a number of factors. In general, however, any experimental design would have as its foundation the four principles of replication, randomisation, cross-classification, and experimentation with similar materials. *Replication* refers to the repetition of the same treatment on different experimental units. *Randomisation* is the use of a random process to assign experimental

units to treatments. *Cross-classification* involves a method of permitting each unit of experimental material to be employed for every treatment under test. The term '*experimental material*' has a broad interpretation and it varies from one experiment to another. In any event, by *similar blocks of experimental materials* we mean that the characteristics of each experimental unit remain approximately constant from one trial to another.

Replication. As pointed out earlier, replication means '*the repetition of the treatments under investigation*'. An experimenter resorts to replication in order to average out the influence of the chance factors on different experimental units. Thus, the repetition of treatments results in more reliable estimate than is possible with a single observation. The following are the chief *advantages* of replication :

At the first instance replication serves to reduce experimental error and thus enables us to obtain more precise estimates of the treatment effects. From statistical theory we know that the standard error (S.E.) of the mean of a sample of size n is $\sigma/\sqrt{n}$, where σ is the standard deviation (per unit) of the population. Thus if a treatment is replicated r times, then the S.E. of its mean effect is $\sigma/\sqrt{r}$, where σ^2, the variance of the individual plot is estimated from the 'error variance'. Thus "*the precision of the experiment is inversely proportional to the square root of the replications*". Consequently replication has an important but limited role in increasing the efficiency of the design.

Remarks **1.** The percentage S.E. to which the mean effect of a treatment replicated r times is :

$$100\left[\left(\sigma/\sqrt{n}\right)-\left(1/\bar{x}\right)\right]=\left[100\left(\sigma/\bar{x}\right)\right]\left(1/\sqrt{n}\right)=\left[C.V./\sqrt{n}\right] \qquad \ldots (6\cdot3)$$

where *C.V.* is the coefficient of variation.

If $\bar{x}_1$ and $\bar{x}_2$ are the mean effects of two treatments replicated r_1 and r_2 times respectively, then

$$\text{Var}\,(\bar{x}_1-\bar{x}_2)=\text{Var}\,(\bar{x}_1)+\text{Var}\,(\bar{x}_2),$$

covariance term vanishes, since $\bar{x}_1$ and $\bar{x}_2$ are independent.

$$\therefore \qquad \text{Var}\,(\bar{x}_1-\bar{x}_2)=\sigma^2\left[(1/r_1)+(1/r_2)\right]$$

where σ^2 as usual is estimated from 'error variance'.

$$\therefore \qquad \left.\begin{aligned} S.E.\,(\bar{x}_1-\bar{x}_2) &= \sigma\left[(1/r_1)+(1/r_2)\right]^{1/2} \\ &= \sigma\sqrt{2/r}\,, \text{ if } r_1=r_2=r, \text{ (say)}\end{aligned}\right\} \qquad \ldots (6\cdot4)$$

An approximate idea of the minimum number of replications required to detect the given difference between two treatments at certain level of significance is obtained by applying normal test to the statistic :

$$t=\frac{\bar{x}_1-\bar{x}_2}{S.E.\,(\bar{x}_1-\bar{x}_2)}=\frac{\bar{x}_1-\bar{x}_2}{\sigma\sqrt{(2/r)}} \qquad \ldots (6\cdot4a)$$

for a large number of degrees of freedom (*see* Example 6·1).

However, for small degrees of freedom, t is not normally distributed but follows Student's t distribution. In that case the (approximate) minimum number of replications required to detect a specified difference 'd' between the treatments at α% level of significance is given by the formula :

$$r=\frac{2\,t_\nu(\alpha/2)\,S_E^2}{d^2}, \qquad \ldots (6\cdot4b)$$

where s_E^2 is the error variance per unit and $t_\nu(\alpha)$ is the right-tail critical value of t at 'α' level of significance and ν = Error degrees of freedom so that

$$P[t>t_\nu(\alpha)]=\alpha \qquad \ldots (6\cdot4c)$$

From Table *VI* in the Appendix, we observe that the critical (significant) values of t decrease as v, the degrees of freedom increases. For example, we have

$t_1(0{\cdot}025) = 12{\cdot}71$; $t_6(0{\cdot}025) = 2{\cdot}45$; $t_{15}(0{\cdot}025) = 2{\cdot}13$; $t_{25}(0{\cdot}025) = 2{\cdot}06$; $t_\infty(0{\cdot}025) = 1{\cdot}96$

Thus the increase in the number of replications will result in increased accuracy, since in that case even small differences will be detected.

2. The most important purpose of replication is to provide an estimate of the experimental error without which we cannot

(*i*) test the significance of the difference between any two treatments, or

(*ii*) determine the length of the confidence interval.

The estimate of the experimental error is obtained by considering the differences in the plots receiving the same treatment in different replications and there is no other alternative of obtaining this estimate.

3. It is desirable to have as much uniformity or homogeneity as possible *within* each *replication* but it is not important to have a great deal of uniformity *between replications.*

4. The adequate number of replications for various treatments in an experiment depends upon the knowledge of the variability of the experimental material, *e.g.,* fertility of soil in field experimentation, which is rarely known and as such cannot be suggested in advance. A general rule is to get as many replications which will provide at least 12 degrees of freedom for the error. This follows from the fact that the values of F-statistic do not decrease rapidly beyond $v_2 = 12$. Usually one should not use less than 4 replications.

Example 6·1. *Calculate the minimum number of replications so that an observed difference of 10% of the mean will be taken as significant at 5% level, the C.V. of the plot values being 12%.*

Solution. If r is the required number of replications and μ be the common mean then we are given : C.V. $= 12 = 100.\ \frac{\sigma}{\mu} \Rightarrow \sigma = \frac{12\mu}{100} = 0{\cdot}12\mu$; and $\bar{x}_1 - \bar{x}_2 = 10\%$ of $\mu = 0{\cdot}10\mu$

$$\therefore \qquad t = \frac{\bar{x}_1 - \bar{x}_2}{\sigma\sqrt{2/r}} = \frac{0{\cdot}10\mu}{0{\cdot}12\mu\sqrt{2/r}}$$

For t to be significant at 5% level of significance, we should have (for large *d.f.*)

$$|t| > 1{\cdot}96 \Rightarrow \frac{10}{12}\sqrt{r/2} > 1{\cdot}96 \Rightarrow r > 2(1{\cdot}96 \times 1{\cdot}2)^2 = 11{\cdot}1$$

Hence, the required (minimum) number of replications is 12 (the next integer greater than 11·1).

Randomisation. As discussed earlier, by replication the experimenter tries to average out as far as possible the effects due to uncontrolled factors. This brings to him the question of allocation of treatments to experimental units so that each treatment gets an equal chance of showing its worth. In the absence of the prior knowledge of the variability of the experimental material, this objective is achieved through '*randomisation*', a process of assigning the treatments to various experimental units in a purely chance manner. The following are the main *objectives* of randomisation :

(*i*) The validity of the statistical tests of significance, *e.g.*, the t-test for testing the significance of the difference of the difference of two means or the 'Analysis of Variance' F-test for testing the homogeneity of several means, depends on the fact that the statistic under consideration obeys some statistical distribution. Randomisation provides a logical basis for that and makes it possible to draw rigorous inductive inferences by the use of statistical theories based on probability theory. This assumption of randomness is necessary since S.E. $(\bar{x}) = \sigma/\sqrt{n}$, for random sampling only. Randomising the treatments over the

experimental units is an essential safeguard against distortion of experimental results by un-anticipated influences such as rise in ambient temperature, drift in calibration of instruments and equipment, fertility of the soil or other systematic changes.

(*ii*) The purpose of randomness is to assure that the sources of variation, not controlled in the experiment, operate randomly so that the average effect on any group of units is zero. In other words, randomisation ensures that different treatments, by the repetition of the experiment, on the average are subject to equal environmental effect. Randomisation eliminates bias in any form. It equalises even factors of variation over which we have no control.

Remark. It should be noted that randomisation without replication is not sufficient. It is only when randomisation of treatments to various units is accompanied by an adequate number of replications then we are in a position to apply the tests of significance (*t*-test or *F*-test).

Local Control. If the experimental material, say field for agriculture experimentation, is heterogeneous and different treatments are allocated to various units (plots) at random over the entire field, the soil heterogeneity will also enter the uncontrolled factors and thus increase the experimental error. It is desirable to reduce the experimental error as far as practicable without unduly increasing the number of replications or without interfering with the statistical requirement of randomness, so that even smaller differences between treatments can be detected as significant. In addition to the principles of replication and randomisation discussed earlier, the experimental error can further be reduced by making use of the fact that neighbouring areas in a field are relatively more homogeneous than those widely spread. In order to separate the soil fertility effects from the experimental error, the whole experimental area (field) is divided into homogeneous groups (blocks) row-wise or column-wise (one-way elimination of fertility gradient, *c.f.* Randomised Block Design) or both (elimination of fertility gradient in two perpendicular directions *c.f.* Latin Square Design, according to the fertility gradient of the soil such that the variation within each block is minimum and between the blocks is maximum. The treatments are then allocated at random within each block. (*The process of reducing the experimental error by dividing the relatively heterogeneous experimental area (field) into homogeneous blocks (due to physical contiguity as far as field experiments are concerned)* is known as *Local Control.*)

Remarks **1.** Local control, by reducing the experimental error, increases the efficiency of the design.

2. Various forms of arranging the units (plots) into homogeneous groups (blocks) have so far been evolved and are known as *experimental designs, e.g.,* Randomised Block Design, Latin Square Design, etc., which are discussed in § 6·6 and § 6·7, etc.

Size of the Plot. The size of the plot depends on a number of factors such as the total experimental area available, the number of treatments, the number of replications of each treatment, the crop, and so on. If the total experimental area remains fixed, then an increase in the size of the plot will result in decrease in the number of plots and consequently result in an increase in the size of the block and decrease in the number of blocks. While deciding about the number of plots, it should also be kept in mind that an increase in the number of plots increases the *non-experimental* area or the so-called *guard-area* by which we mean the strips of land which are left out between consecutive plots and also between consecutive blocks in order to reduce the flow of the experimental material from one plot to another. Fairfield Smith, after conducting uniformity trial experiments with the same crop and then harvesting the crop in small units, obtained an important empirical relationship between the

plot size and the plot variance. This relationship, known as *Fairfield Smith's Variance Law*, is expressed by the equation :

$$V_x = \frac{V_1}{x^b} \qquad \ldots (6{\cdot}5) \quad \Rightarrow \quad \log V_x = \log V_1 - b \log x \qquad \ldots (6{\cdot}5a)$$

where V_x is the variance of the yield per unit area from plots of size x units, V_1 is the variance among plots of size unity and b, the regression coefficient, is a soil characteristic indicating the relationship between adjacent units. The limiting values of b are 1 and 0. $b = 1$ means that experimental unit is composed of a random selection of x individuals, *i.e.*, the units making the plot of size x units are not correlated. In this case ($b = 1$) , we get from (6·5)

$$V_x = \frac{V_1}{x}, \qquad \ldots (6{\cdot}5b)$$

so that the precision of the experiment increases with an increase in the plot size. In field experimentation, the adjacent areas of land are usually correlated and thus the value of b will be less than unity. $b = 0$ means that the x units of the plot are perfectly correlated and in this case we get from (6·5) $V_x = V_1$, meaning thereby that in such a situation, the increase in the plot size does not result in any gain in efficiency.

TABLE 6·1

Name of the crop	*Plot size in acres*
Cereals	1/10
Maize	1/20
Sugarcane	1/40 to 1/20
Vegetables	1/80

Remark. *The Table 6·1.* gives the optimum sizes of plots for different crops :

Shape of Blocks and Plots. The shape and size of the blocks will usually depend upon the shape and size of the plots. In order to control the experimental error, it is desirable to divide the whole experimental area into different sub-groups (blocks) such that within each block there is as much homogeneity as possible but between blocks there is maximum variation. Further each block is to be divided into as many plots as the number of treatments. For maximum precision the plots should be rectangular in shape with their long sides parallel to the direction of the fertility gradient and the blocks should be arranged one after the other along the fertility gradient as shown in the *Fig. 6·2.*

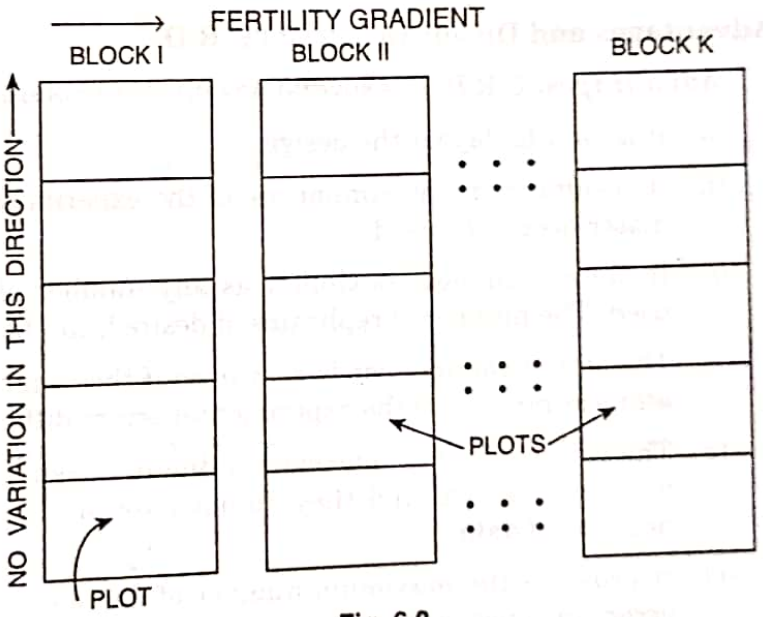


Fig. 6·2

In the following sequences, we shall discuss the designing and the analysis of some of the important designs of experiments.

6·4. COMPLETELY RANDOMISED DESIGN (C.R.D.)

The simplest and most flexible design is the completely randomised design. In this design the experimental units are allotted at random to the treatments, so that every unit gets the

same chance of receiving every treatment. In addition the units should be processed in random order at all subsequent stages in the experiment where this order is likely to affect the results. Also in this design treatments are allocated at random to the experimental units over the entire experimental material. Let us suppose that we have v treatments, the ith treatment being replicated r_i times, $i = 1, 2, \ldots, v$. Then the whole experimental material is divided into $n = \Sigma r_i$ experimental units and the treatments are distributed completely at random over the units subject to the condition that the ith treatment occurs r_i times. Randomisation assures that extraneous factors do not continually influence one treatment. In particular case if $r_i = r \forall i = 1, 2, \ldots, v$, *i.e.,* if each treatment is repeated an equal number of times r, then $n = rv$ and randomisation gives every group of r units an equal chance of receiving the treatments. In general, equal number of replications for each treatment should be made expect in particular cases when some treatments are of greater interest than others or when practical limitations dictate otherwise. Also a table of random numbers is to be used to assign the units to the treatments. For instance, suppose there are v treatments to be compared and we have n experimental units available. Let the ith treatment be replicated r_i times so that $n = \sum_i r_i$. The treatments may be numbered arbitrarily from 1 to v, and the experimental units from 1 to n. r_1 units selected at random from the n units, using a table of random numbers, may be allocated to the first treatment ; r_2 units selected randomly from the remaining units to the second treatment, and so on.

Advantages and Disadvantages of C.R.D.

Advantages. C.R.D. has several advantages explained below :

(*i*) It is easy to layout the design.

(*ii*) It results in the maximum use of the experimental units since all the experimental materials can be used.

(*iii*) It allows complete flexibility as any number of treatments and replicates may be used. The number of replicates, if desired, can be varied from treatment to treatment.

(*iv*) The statistical analysis is easy even if the number of replicates are not the same for all treatments or if the experimental errors differ from treatment to treatment.

(*v*) The relative loss of information due to missing data is smaller in comparison with any other design and they do not pose any problem in carrying out the standard analysis of data.

(*vi*) It provides the maximum number of degrees of freedom for the estimation of the error variance, which increases the sensitivity or the precision of the experiment for small experiments, *i.e.,* for experiments with small number of treatments.

Disadvantages. (*i*) In certain circumstances, the design suffers from the disadvantage of being inherently less informative than other more sophisticated layouts. This usually happens if the experimental material is not homogeneous.

(*ii*) Since randomisation is not restricted in any direction to ensure that the units receiving one treatment are similar to those receiving the other treatment, the whole variations among the experimental units is included in the residual variance. This makes the design less efficient and results in less sensitivity in detecting significant effects. As such *C.R.D.* is seldom used in *field experimentation,* where due to the fertility gradient of the soil

the whole experimental material, *viz.*, field, is not homogeneous and it is better to use more efficient designs like Randomised Block Design (R.B.D.) or Latin Square Design (L.S.D.), *etc.* discussed in § 6·6 and § 6·7, etc.

Applications. (*i*) Completely randomised design is most useful in laboratory technique and methodological studies, *e.g.*, in physics, chemistry or cookery, in chemical and biological experiments, in some green house studies, etc., where either the experimental material is homogeneous or the intrinsic variability between units can be reduced.

(*ii*) C.R.D. is also recommended in situations where an appreciable fraction of units is likely to be destroyed or fail to respond.

6·4·1. Statistical Analysis of C.R.D. Statistical analysis of a C.R.D. is analogous to the ANOVA for a one-way classified data for fixed effect model, the linear model (assuming various effects to be additive) becomes

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij},\ i = 1, 2, \ldots, v\ ; j = 1, 2, \ldots, r_i \qquad \ldots (6\text{·}6)$$

where y_{ij} is the yield or response from the jth unit receiving the ith treatment, μ is the general mean effect, τ_i is the effect due to the ith treatment, where μ and τ_i are constants so that $\sum_{i=1}^{n} r_i \tau_i = 0$ and ε_{ij} is error effect due to chance such that ε_{ij} is identically and independently distributed (*i.i.d.*) $N(0, \sigma_e^2)$. Then, $n = \sum_{i=1}^{n} r_i$ is the total number of experimental units.

The analysis of the model (6·6) is same as that of one-way classified data for fixed effect model discussed in § 5·2·1. If we write : $\sum_i \sum_j y_{ij} = y.. = G$ = Grand total of all the n observations, and

$\sum_{j=1}^{r_i} y_{ij} = y_{i\cdot} = T_i$ = Total response in the units receiving the ith treatment, then, as in ANOVA (one-way classified data),

$$\sum_{i=1}^{v} \sum_{j=1}^{r_i} \left(y_{ij} - \bar{y}..\right)^2 = \sum_i \sum_j \left(y_{ij} - \bar{y}_{i\cdot}\right)^2 + \sum_{i=1}^{v} r_i \left(\bar{y}_{i\cdot} - \bar{y}..\right)^2$$

i.e.

$$T.S.S. = S.S.E. + S.S.T.$$

where *T.S.S.*, *S.S.T.* and *S.S.E.* are the total sum of squares, sum of squares due to treatments (between treatments *S.S.*) and sum of squares due to error (*i.e.*, within treatment *S.S.*) given respectively by

$$\left.\begin{aligned} T.S.S. &= \sum_i \sum_j \left(y_{ij} - \bar{y}..\right)^2, \\ S.S.T. &= \sum_i r_i \left(\bar{y}_{i\cdot} - \bar{y}..\right)^2 = S_T^2 \text{ (say)} \\ S.S.E. &= \sum_i \sum_j \left(y_{ij} - \bar{y}_{i\cdot}\right)^2 = S_E^2 \text{ (say)} \end{aligned}\right\} \qquad \ldots (6\text{·}7)$$

The statistical analysis for such a design can be carried out exactly similarly as discussed in § 5·2·4 [ANOVA for Random Effect Model for One-way Classified Data] with k replaced by v, with unequal classes n_i by r_i and N by $n = \sum_i r_i$.

The null hypothesis of interest will be : $H_0 : \sigma_\tau^2 = 0$ *i.e.*, all the class means are equal.

Remark. For the random effect model (6·15), the expectations of the various *S.S.* are [*c.f.* (5·27) and (5·29)] :

$$E\left(\frac{SST}{t-1}\right) = E\,(MST) = \sigma_e^2 + r\,\sigma_\tau^2 \qquad \ldots (6\text{-}16a)$$

$$E\left(\frac{SSE}{rt-t}\right) = E\left[\frac{SSE}{t(r-1)}\right] = E(MSE) = \sigma_e^2 \qquad \ldots (6\text{-}16b)$$

6·5. RANDOMISED BLOCK DESIGN (R.B.D.)

In field experimentation, if the whole of the experimental area is not homogeneous and the fertility gradient is only in one direction, then a simple method of controlling the variability of the experimental material consists in stratifying or grouping the whole area into relatively homogeneous strata or sub-groups (or *blocks* or *replicates*, as they are called), perpendicular to the direction of the fertility gradient. Now if the treatments are applied at random to relatively homogeneous units within each strata or block and replicated over all the blocks, the design is a *Randomised Block Design* (*R.B.D.*).

In a *C.R.D.*, we do not resort to the grouping of the experimental site (space, material or time) and allocate the treatments at random to the experimental units. But in *R.B.D.* treatments are allocated at random within the units of each stratum or block, *i.e.*, randomisation is restricted. Also variation among blocks is removed from variation due to error. Hence, if it is desired to control one source of variation by stratification, the experimenter should select the *R.B.D.* rather than *C.R.D.*

Remarks **1.** Since all the treatments are to be applied within each block, in each block we take as many units (or plots) as the number of treatments. With this design each treatment will have the same number of replications. If we want additional replications for some treatments, each of them must be applied to more than one unit in a block.

2. It is assumed that though the general level of results is different in the different blocks, the relative effects of treatments are same in all the blocks apart from experimental error. In other words, there is no interaction between treatments and blocks. In practice we interpret this as meaning that the interactions, if any exist, are not appreciable compared with the treatment effects. Interactions will be separable from experimental error and if the interactions are large, the experiment may yield misleading results.

3. Since its development (about 1925) *R.B.D.* has become extremely popular in a large number of fields. It is flexible, readily adaptable and easy to analyse and these points have made it the most popular of all the designs with Latin Square Design (*L.S.D.*) (*c.f.* § 6·7) being its closest rival.

4. *R.B.D.* provides a method of eliminating or reducing the effects of trends. The design is not limited to experiments in fields or experiments in industrial plants alone but applies whenever the trials are spread over a period of time or space and the possibility of systematic variation or trends exists.

5. By means of grouping, the experimental error is reduced and the treatment comparisons are made more sensitive than with completely randomised designs.

6. Any number of treatment and any number of replicates may be included. However, if the number of treatments is large (20 or more) the efficiency of error control decreases and then there are other designs that are more efficient than R.B.D.

7. When data from some individual units are missing, the 'missing plot' technique discussed in § 6·8 enables the available results to be fully utilised. Some extra computational labour is, however, involved, and if the gaps are numerous the design is less convenient in this respect than *C.R.D.*

Applications of R.B.D. Despite its agricultural origin, the randomised-blocks design is widely used in many types of studies today. For instance, to determine the differences in productivity of, say, *c* makes of machines (treatments), we may isolate the possible effects due to differences in efficiency among operators (blocks) by randomly selecting operations and then randomly rotating machine assignments in such a way that each operator works on all the machines. The basic idea here is to compare treatment levels (the different machines) within a block of relatively homogeneous experimental material (the same operator), then repeat the comparison on another block (another operator) , and so on for additional repetitions of the comparison. The primary difference between the preceding model and the present one lies in the manner in which various experimental units are assigned. In the completely randomized design there are no blocks each of which must receive every treatment level; there are no restrictions on assigning experimental units to treatment levels. In the randomised blocks model the sub-divisions of blocks of experimental units are randomly assigned to the treatments after the blocks have been deliberately arranged to be homogeneous.

Layout of R.B.D. In agricultural experimentation, the layout of *R.B.D.* can be illustrated as follows :

Let us consider five treatments *A, B, C, D and E* each replicated four times. We divide the whole experimental area into four relatively homogeneous strata or blocks and each block into five units or plots. Treatments are then allocated *at random* to the plots of a block, fresh randomisation being done for each block. A particular layout may be as follows :

Block	I	*A*	*E*	*B*	*D*	*C*
Block	II	*E*	*D*	*C*	*B*	*A*
Block	III	*C*	*B*	*A*	*E*	*D*
Block	IV	*A*	*D*	*E*	*C*	*B*

For randomisation, we may use Tippet's random number tables. Let us select one digited numbers in the order of their occurrence in the table leaving zero and numbers greater than 5. Suppose we get a random permutation of the digits from 1 to 5 as : 1, 3, 5, 4, 2. So in the first block we allocate treatment *A* to first plot, *B* to 3rd, *C* to 5th, *D* to 4th and *E* to 2nd plot. Similarly, we find fresh random permutations for each of the other three blocks and allocate the treatments accordingly.

Advantages of R.B.D. Chief advantages of R.B.D. may be outlined as follows :

(*i*) *Accuracy.* This design has been shown to be more efficient or accurate than C.R.D. for most types of experimental work. The elimination of between S.S. from residual S.S., usually results in a decrease of error mean S.S.

(*ii*) *Flexibility.* In R.B.D. no restrictions are placed on the number of treatments or the number of replicates. In general, at least *two* replicates are required to carry out the test of significance (Factorial design is an exception). In addition, control (check) or some other treatments may be included more than once without complications in the analysis.

(*iii*) *Ease of analysis.* Statistical analysis is simple, rapid and straightforward. Moreover the error of any treatment can be isolated and any number of treatments may be omitted from the analysis without complicating it.

[Also see Remarks 3 to 7 to § 6·5.]

Disadvantages of R.B.D.

(*i*) *R.B.D.* may give misleading results if blocks are not homogeneous.

(*ii*) *R.B.D.* is not suitable for large number of treatments because in that case the block size will increase and it may not be possible to keep large blocks homogeneous.

(*iii*) If the data on more than two plots is missing, the statistical analysis becomes quite tedious and complicated.

6·5·1. Statistical Analysis of R.B.D. for One Observation Per Experimental Unit. If in an R.B.D. a single observation is made on each of the experimental units, then its analysis is analogous to ANOVA for fixed effect model for a two-way classified data with one observation per cell [*c.f* § 5·3·1] and the linear model (assuming various effects to be additive) becomes :

$$y_{ij} = \mu + \tau_i + b_j + \varepsilon_{ij} \; ; (i = 1, 2, \ldots, t \; ; j = 1, 2, \ldots, r) \qquad \ldots (6\cdot17)$$

where

y_{ij} is the response or the yield of the experimental unit receiving the ith treatment in the jth block ;

μ is the general mean effect ;

τ_i is the effect due to the ith treatment;

b_j is the effect due to jth block or replicate

and $\varepsilon_{ij} \overset{i.i.d}{\sim} N(0, \sigma_e^2)$,

where μ, τ_i's and b_j's are constants so that $\sum_{i=1}^{t} \tau_i = 0$ and $\sum_{i=1}^{r} b_j = 0$ $\qquad \ldots (6\cdot17a)$

If we write :

$\sum_i \sum_j y_{ij} = y.. = G$ = grand total of all the $t \times r$ observations.

$\sum_j y_{ij} = y_{i\cdot} = T_i$ = Total for ith treatment.

$\sum_i y_{ij} = y_{\cdot j} = B_j$ = Total for jth block,

then heuristically, we get

$$\sum_i \sum_j \left(y_{ij} - \bar{y}..\right)^2 = \sum_i \sum_j \left[(\bar{y}_{i\cdot} - \bar{y}..) + (\bar{y}_{\cdot j} - \bar{y}..) + (y_{ij} - \bar{y}_{i\cdot} - \bar{y}_{\cdot j} + \bar{y}..)\right]^2$$

$$= r \sum_i (\bar{y}_{i\cdot} - \bar{y}..)^2 + t \sum_j (\bar{y}_{\cdot j} - \bar{y}..)^2 + \sum_i \sum_j (y_{ij} - \bar{y}_{i\cdot} - \bar{y}_{\cdot j} + \bar{y}..)^2$$

the product terms vanish since the algebraic sum of deviations from mean is zero. Thus

$$T.S.S. \quad S.S.T. + S.S.B. + S.S.E.$$

where *T.S.S.* is the total sum of squares and *S.S.T.*, *S.S.B.* and *S.S.E.* are the sum of squares due to treatments, blocks and errors respectively, give by

$$T.S.S. = \sum_i \sum_j (y_{ij} - \bar{y}..)^2, \qquad S.S.T. = S_T^2 = r \sum_i (\bar{y}_{i\cdot} - \bar{y}..)^2$$

$$S.S.B. = S_B^2 = t \sum_j (\bar{y}_{\cdot i} - \bar{y}..)^2, \qquad S.S.E. = S_E^2 = T.S.S. - S.S.T. - S.S.B.$$

Hence the total sum of squares is partitioned or split into three sum of squares whose degrees of freedom add to the degrees of freedom of *T.S.S.* Therefore, by Cochran's theorem, each of these S.S. divided by σ_e^2 is independently distributed as χ^2-variate.

TABLE 6·10 : ANOVA TABLE FROM R.B.D.

Source of variation	*d.f.*	*S.S.*	*M.S.S.*	*Variance Ratio*
Treatments	$t-1$	S_T^2	$s_T^2 = S_T^2/(t-1)$	$F_T = s_T^2/s_E^2$
Blocks or Replicates	$r-1$	S_B^2	$s_B^2 = S_B^2/(r-1)$	$F_B = s_B^2/s_E^2$
Error or Residual	$(t-1)(r-1)$	S_E^2	$s_E^2 = S_E^2/(t-1)(r-1)$	
Total	$rt-1$			

M.S.S. of treatments and replicates (or blocks) are tested for significance against error mean *S.S.*

Under the null hypothesis $H_{ot}: \tau_1 = \tau_2 = \ldots = \tau_t$ against the alternative that all τ's are not equal the test statistic is :

$$F_T = \frac{s_T^2}{S_E^2} \sim F[(t-1), (t-1)(r-1)]$$

i.e., F_T follows F(central) distribution with $[(t-1), (t-1)(r-1)]$ *d.f.* Thus if F_T is greater than tabulated F for $[(t-1), (t-1)(r-1)]$, *d.f.* at certain level of significance, usually 5%, then we reject the null hypothesis H_{ot} and conclude that the treatments differ significantly. If F_t is less than tabulated value then F_T is not significant and we conclude that the data do not provide any evidence against the null hypothesis which may be accepted.

Similarly under the null hypothesis $H_{ob}: b_1 = b_2 = \ldots = b_r$, against the alternative that b's are *not* all equal, the test statistic is :

$$F_B = \frac{s_B^2}{s_E^2} \sim F[(r-1), (r-1)(t-1)]$$

and we can discuss its significance as explained above.

Remarks **1.** The following formulae reduce arithmetic to a great extent for the calculation of various S.Ss.

Let us write : $$G = \sum_i \sum_j y_{ij} \text{ and } N = rt \quad \ldots (6\cdot18)$$

$$\text{Raw } S.S.\ (R.S.S.) = \sum_i \sum_j y_{ij}^2 \text{ ; Correction Factor } (C.F.) = \frac{G^2}{N} \quad \ldots (6\cdot18a)$$

$$\text{Total } S.S. = T.S.S. = \sum_i \sum_j (y_{ij} - \bar{y}..)^2 = R.S.S. - C.F. \quad \ldots (6\cdot19)$$

$$S.S. \text{ due to Treatments} = S.S.T = r \sum_i (\bar{y}_{i\cdot} - \bar{y}..)^2 = \frac{\sum_i y_{i\cdot}^2}{r} - C.F. = \frac{\sum_i T_i^2}{r} - C.F. \quad \ldots (6\cdot20)$$

where $y_{i\cdot} = T_i = \sum_{j=1}^{r} y_{ij}$, is the total yield from the ith treatment

$$S.S. \text{ due to Blocks} = S.S.B. = \frac{\sum_j y_{\cdot j}^2}{t} - C.F. = \frac{\sum_j B_j^2}{t} - C.F. \quad \ldots (6\cdot21)$$

where $y_{\cdot j} = B_j = \sum_{i=1}^{t} y_{ij}$, is the total yield from the jth block

$$S.S. \text{ due to Error} = S.S.E. = T.S.S. - S.S.T. - S.S.E. \quad \ldots (6\cdot22)$$

Thus we see that neither F_t nor F_b are significant and consequently H_{ob} and H_{hb} may be retained, *i.e.*, we may regard the treatments as well as blocks to be homogeneous.

Remarks **1.** Here one degree of freedom is lost for total S.S. and consequently for error S.S. due to the estimation of the missing value from the given data.

2. If the hypothesis $H_t : \tau_1 = \tau_2 = \tau_3 = \ldots \tau_t$ had been rejected, then we would have proceeded further for finding which pair of treatments differ significantly, as discussed in Remark 2 § 6·6·1. and using (6·39) for treatment pairs one of which contains the missing observation.

6·6. LATIN SQUARE DESIGN (L.S.D.)

In R.B.D. whole of the experimental area is divided into relatively homogeneous groups (blocks) and treatments are allocated at random to units within each block, *i.e.*, randomisation was subjected to one restriction, *i.e.*, within blocks. But in field experimentation, it may happen that experimental area (field) exhibits fertility in strips, *e.g.*, cultivation might result in alternative strips of high or low fertility. R.B.D. will be effective if the blocks happen to be parallel to these strips and would be extremely inefficient if the blocks are across the strips. Initially, fertility gradient is seldom known. A useful method of eliminating fertility variations consists in an experimental layout which will control variation in two perpendicular directions. Such a layout is a *Latin Square Design* (*L.S.D.*)

Remark. The Latin square differs from the randomised block design in that the treatments are arranged in complete groups in two directions, the two classifications being orthogonal to each other and to the treatments. In a Latin square every row and every column is a complete replication. The effect of the double grouping is to eliminate from the errors all differences among rows and equally all differences among columns. Thus, the Latin square provides more opportunity than randomised blocks for the reduction of errors by skillful planning. The experimental material should be arranged and the experiment conducted so that the differences among rows and columns represent major sources of variation.

Layout of Design. In field-plot experiments, the Latin square is usually laid out in the conventional square with the rows and columns corresponding to possible fertility trends in two directions across the field. In other types of experiments, the rows and columns may be made to correspond to different sources of error as in an animal feeding experiment where the column groups may correspond with initial weight and the row group with age.

In this design the number of treatments is equal to the number of replications. Thus in case of m treatments, there have to be $m \times m = m^2$ experimental units. The whole of experimental area is divided into m^2 experimental units (plots) arranged in a square so that each row as well as each column contains m units (plots). The m treatments are then allocated at random to these rows and columns in such a way that every treatment occurs once and only once in each row and in each column. Such a layout is known as $m \times m$ *Latin Square Design* (*L.S.D.*) and is extensively used in agricultural experiments. For example, if we are interested in studying the effects of m types of fertilisers on the yield of a certain variety of wheat, it is customary to conduct the experiments on a square field with m^2-plots of equal area and to associate treatments with different fertilisers and row and column effects with variations in fertility of soil.

Obviously, there can be many arrangements for an $m \times m$ L.S.D. and a particular layout in an experiment must be determined randomly.

Standard Latin Square. A Latin square in which the treatments, say, *A, B, C* ... *etc.* occur in the first row and the first column in alphabetical order is called a *Standard Latin Square* or a *Latin Square in Canonical Form.*

For 2 × 2 and 3 × 3 Latin squares, only one standard square exists; as given in *Fig. 6·3* and *Fig. 6·4.*

For a 4 × 4 Latin Square Design, 4 standard squares are possible as given in *Figures 6·5a to 6·5d.*

A	B
B	A

2 × 2 Standard *LSD*

Fig. 6·3

A	B	C
B	C	A
C	A	B

3 × 3 Standard *LSD*

Fig. 6·4

4 × 4 STANDARD LATIN SQUARE DESIGNS

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

Fig. 6·5(*a*)

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

Fig. 6·5(*b*)

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

Fig. 6·5(*c*)

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

Fig. 6·5(*d*)

From a standard Latin square, we can generate a number of Latin squares by permuting the rows, columns and treatments, which are known as *transformation sets* of Latin squares. The number of squares that can be generated from a standard $m \times m$ Latin square by permuting the rows, columns and letters (treatments) is $(m\,!)^3$. These are not necessarily all different. If all rows except the first and all columns are permuted, we generate $[m\,! \times (m-1)\,!]$ squares.

Total number of possible Latin squares of order $m \times m$

$\therefore$ $= m\,! \times (m-1)\,! \times$ [Number of standard squares] ... (6-40)

The following *Table 6·21* gives the total number of $m \times m$ Latin squares that can be constructed for different values of m.

TABLE 6·21 : TOTAL NUMBER OF LATIN SQUARES

m	*Number of Standard Squares* (k)	$m\,!\,(m-1)\,!$	*Total No. of Latin Squares* $m\,!\,(m-1!\times k$
3	1	$6 \times 2 = 12$	12
4	4	$24 \times 6 = 144$	576
5	56	$120 \times 24 = 2880$	1,61,280
6	9,408	$720 \times 120 = 86400$	81,285 1,200

Thus we see that the total number of possible arrangements increases very rapidly as the value of m (the size of Latin square) increases, thus making it rather impossible to tabulate all such arrangements for higher values of m. ***Fisher and Yates*** have tabulated some Standard Latin Squares in Tables *XV* and *XVI* of *Fisher and Yates' Statistical Tables* for Biological, Agricultural and Medical Research.

Remark. Thus in the Latin square matrix each 'treatment' appears once in each row and once in each column. Randomisation of the treatments, however, is basic to this design. Therefore, tables of random numbers are often used in assigning of the treatments to the rows and columns, always providing that each treatment appears but once in each row and each column. A table of random numbers becomes particularly useful in a 7 × 7 or 8 × 8 Latin square. The total number of possible arrangements depends upon the size of the square. From the *Table 6·21.*, we observe that in a 3 × 3

Latin square there are 12 possible arrangements and in 4 × 4 Latin square there are 576 possible arrangements. The number of possible arrangements increases very rapidly as n^2 (number of cells) increases. When $n = 5(5 \times 5)$ there are 1,61,280 different squares, when $n = 6$ (6×6) there are some 813 millions. Thus, use of random numbers for selecting the arrangement is very helpful.

Advantages of Latin Square Design (L.S.D.) **1.** With two-way grouping or stratification L.S.D. controls more of the variation than C.R.D. or R.B.D.

The two-way elimination of variation as a result of cross grouping often results in small error mean sum of squares. Thus, in field experimentation if the fertility gradient is in two directions at right angles to each other (*i.e.*, if there is a diagonal trend in fertility) or in one unknown direction then L.S.D. is likely to be more efficient than R.B.D. In fact L.S.D. can be used with advantage of those cases where the variation in experimental material is from two orthogonal sources. As regards the applications of L.S.D., Professor Fisher* says, "If experimentations were only concerned with the comparison of four to eight treatments or varieties, it (L.S.D.) would be not merely the principal but almost the universal design employed." In the words of Kenney and Keeping, "The Latin square is a device for controlling two sources of error at once. In field work, the treatments are so allocated among the plots that no treatment occurs more than once in any one row or any one column of the Latin square. Variability among rows and among columns is removed from the error. This serves to control variability due to gradients of soil fertility in two directions at right angles across the field."

2. L.S.D. is an incomplete 3-way layout. Its advantage over the complete 3-way layout is that instead of m^3 experimental units only m^2 units are needed. Thus a 4 × 4 L.S.D. results in saving of $m^3 = 4^3 - 4^2 = 64 - 16 = 48$ observations over a complete 3-way layout

3. The statistical analysis is simple though slightly complicated than for R.B.D. Even with 1 or 2 missing observations the analysis remains relatively simple.

4. More than one factor can be investigated simultaneously and with fewer trials than more complicated designs.

Disadvantages of L.S.D. **1.** The fundamental assumption that there is no interaction between the three factors of variation (*i.e.*, the factors act independently) may not be true in general.

2. Unlike R.B.D., in L.S.D. the number of treatments is restricted to the number of replications and this limits its field of application. L.S.D. is suitable for the number of treatments between 5 and 10 and for more than 10 to 12 treatments the design is seldom used since in that case the square becomes too large and does not remain homogeneous.

3. In case of missing plots, when several units are missing the statistical analysis becomes quite complex. If one or two blocks in a field are attacked by some disease or pest then in R.B.D. we can easily omit the data for these blocks without complicating the analysis at all whereas this is not possible in LSD because in LSD the number of rows, columns and treatments have to be equal. A much more complicated analysis is necessitated in L.S. experiment under similar conditions.

4. In the field layout, R.B.D. is much easy to manage than L.S.D., since the former can be performed equally well on a square or rectangular field or a field of any shape whereas for the latter approximately a square field is necessary.

Remark. It should be emphasised at this point that the Latin square is not suitable when the number of treatments is large as there will be as many replications as there are treatments. Moreover, when we have more treatments, it may be difficult to allocate the rows and the columns to sources of variability in an efficient manner. On the other hand, when the number of treatments is very small,

Latin square does not provide sufficient number of degrees of freedom to give a reliable estimate of error variance.

6·6·1. Statistical Analysis of m × m L.S.D. for One Observation per Experimental Unit. Let y_{ijk} $(i, j, k = 1, 2, \ldots, m)$ denote the response from the unit (plot, in field experimentation) in the ith row, jth column and receiving the kth treatment. The triple (i, j, k) assumes only m^2 of the possible m^3 values of an L.S. selected by the experiment. If S represents the set of m^2 values, then symbolically $(i, j, k) \in S$. If a single observation is made per experimental unit, then the linear additive model is :

$$y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + \varepsilon_{ijk}, (i, j, k) \in S \quad \ldots (6\cdot41)$$

where μ is the constant mean effect; α_i, β_j and τ_k are the constant effects due to the ith row, jth column and kth treatment respectively and ε_{ijk} is error effect due to random component assumed to be normally distributed with mean zero and variance σ_e^2 *i.e.*, $\varepsilon_{ijk} \overset{i.i.d}{\sim} N(0, \sigma_e^2)$. If we write

$G = y\ldots$ = Total of all the m^2 observations
$R_i = y_{i\cdot\cdot}$ = Total of the m observations in the ith row
$C_j = y_{\cdot j\cdot}$ = Total of the m observations in the jth column
$T_k = y_{\cdot\cdot k}$ = Total of the m observations from kth treatment,

then heuristically, we have

$$\sum_{i,j,k \in S} (y_{ijk} - \bar{y}\ldots)^2 = \sum_{i,j,k \in S} [(\bar{y}_{i\cdot\cdot} - \bar{y}\ldots) + (\bar{y}_{\cdot j\cdot} - \bar{y}\ldots) + (\bar{y}_{\cdot\cdot k} - \bar{y}\ldots) + (y_{ijk} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} - \bar{y}_{\cdot\cdot k} + 2\bar{y}\ldots)]^2$$

$$= m \sum_i (\bar{y}_{i\cdot\cdot} - \bar{y}\ldots)^2 + m \sum_j (\bar{y}_{\cdot j\cdot} - \bar{y}\ldots)^2 + m \sum_k (\bar{y}_{\cdot\cdot k} - \bar{y}\ldots)^2 + \sum_{i,j,k \in S} (y_{ijk} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} - \bar{y}_{\cdot\cdot k} + 2\bar{y}\ldots)^2$$

the product terms vanish, since the algebraic sum of deviations from mean is zero.

$$\therefore \quad \text{T.S.S.} = \text{S.S.R.} + \text{S.S.C.} + \text{S.S.T.} + \text{S.S.E.}$$

where T.S.S. is the total sum of squares and S.S.R., S.S.C., S.S.T. and S.S.E. represent sum of squares due to rows, columns, treatments and error respectively, given by :

$$\text{T.S.S.} = \sum_{i,j,k \in S} (y_{ijk} - \bar{y}\ldots)^2 ; \quad \text{S.S.R.} = S_R^2 = m \sum_i (\bar{y}_{i\cdot\cdot} - \bar{y}\ldots)^2$$

$$\text{S.S.C.} = S_C^2 = m \sum_j (\bar{y}_{\cdot j\cdot} - \bar{y}\ldots)^2 ; \quad \text{S.S.T.} = S_T^2 = m \sum_k (\bar{y}_{\cdot\cdot k} - \bar{y}\ldots)^2, \text{ and}$$

$$\text{S.S.E.} = S_E^2 = \text{T.S.S.} - \text{S.S.R.} - \text{S.S.C.} - \text{S.S.T.}$$

TABLE 6·19 : ANOVA TABLE FOR $m \times m$ L.S.D.

Source of Variation	*d.f.*	*S.S.*	*M.S.S.*	*Variance Ratio 'F'*
Rows	$m-1$	S_R^2	$s_R^2 = S_R^2/(m-1)$	$F_R = s_R^2 / s_E^2$
Columns	$m-1$	S_C^2	$s_C^2 = S_C^2/(m-1)$	$F_C = s_C^2 / s_E^2$
Treatments	$m-1$	S_T^2	$s_T^2 = S_T^2/(m-1)$	$F_T = s_T^2 / s_E^2$
Error	$(m-1)(m-2)$	S_E^2	$s_E^2 = S_E^2/(m-1)(m-2)$	
Total	m^2-1			

Let us set up Null Hypotheses :

For row effects, $H_{0\alpha}: \alpha_1 = \alpha_2 = \ldots\ldots = \alpha_m = 0$,

For column effects, $H_{0\beta}: \beta_1 = \beta_2 = \ldots\ldots = \beta_m = 0$ and

For treatment effects, $H_{0\tau}: \tau_1 = \tau_2 = \ldots\ldots = \tau_m = 0$.

Alternative Hypotheses :

For row effects, $H_{1\alpha}$: At least two α_i's are different

For column effects, $H_{1\beta}$: At least two β_i's are different

For treatment effects, $H_{1\tau}$: At least two τ_i's are different

The variance ratios F_R, F_C & F_T follow (central) F distribution with $(m-1)$, $(m-1)(m-2)$ *d.f.* under the null hypotheses H_α, H_β and H_τ respectively.

Let $F_\alpha = F_\alpha[(m-1), (m-1)(m-2)]$ be the tabulated value of F for $[(m-1), (m-1)(m-2)]$ *d.f.* at the level of significance 'α'. Thus if $F_R > F_\alpha$ we reject $H_{0\alpha}$ and if $F_R \le F_\alpha$, we fail to reject $H_{0\alpha}$.

Similarly, we can test for $H_{0\beta}$ and $H_{0\tau}$.

Remarks **1.** For numerical computations of various sum of squares, the following formulae are much more convenient to use :

Let us write : $N = m^2$

$G = \sum_{(i,j,k) \in S} y_{ijk}$ = Sum of all the m^2 observations ... (6·42)

$y_{i..} = R_i$ = Sum of the observations from the units in the ith row.

$y_{.j.} = C_j$ = Sum of the observations from the units in the jth column

$y_{..k} = T_k$ = Sum of the observations from the units receiving the kth treatment ... (6·42*a*)

$$\text{Correction Factor } (C.F.) = G^2/N \quad \ldots (6\cdot42b)$$

$$\text{Raw S.S. } (\text{R.S.S.}) = \sum_{(i,j,k) \in S} y_{ijk}^2 \quad \ldots (6\cdot42c)$$

$$\text{T.S.S.} = \sum_{(i,j,k) \in S} (y_{ijk} - \bar{y}_{...})^2 = \text{R.S.S.} - \text{C.F.} \quad \ldots (6\cdot43)$$

$$\text{S.S.R.} = S_R^2 = m \sum_i (\bar{y}_{i..} - \bar{y}_{...})^2 = \frac{1}{m}\sum_i R_i^2 - \text{C.F.} = \frac{1}{m}\sum_i y_{i..}^2 - \text{C.F.} \quad \ldots (6\cdot44)$$

$$\text{S.S.C.} = S_C^2 = m \sum_j (\bar{y}_{.j.} - \bar{y}_{...})^2 = \frac{1}{m}\sum_j C_j^2 - \text{C.F.} = \frac{1}{m}\sum_j y_{.j.}^2 - \text{C.F.} \quad \ldots (6\cdot45)$$

$$\text{S.S.T.} = S_T^2 = m \sum_k (\bar{y}_{..k} - \bar{y}_{...})^2 = \frac{1}{m}\sum_k T_R^2 - \text{C.F.} = \frac{1}{m}\sum_k y_{..k}^2 - \text{C.F.} \quad \ldots (6\cdot46)$$

$$\text{S.S.E.} = \text{T.S.S.} - \text{S.S.R.} - \text{S.S.C.} - \text{S.S.T.} \quad \ldots (6\cdot47)$$

2. Standard Error (S.E.) of the difference between any two treatment means is given by :

$$\text{S.E.}, (\bar{t}_i - \bar{t}_j) = \left\{ S_E^2 \left(\frac{1}{m} + \frac{1}{m} \right) \right\}^{\frac{1}{2}} = \left(\frac{2S_S^2}{m} \right)^{\frac{1}{2}} \quad \ldots (6\cdot48)$$

and the critical difference (C.D.) for the significance of the difference between any two treatment means at level of significance 'α' is given by :

$$\text{C.D.} (\bar{t}_i - \bar{t}_j) = t_{(\text{error d.f.})}(\alpha/2) \times \text{S.E.} (\bar{t}_i - \bar{t}_j)$$
$$= t_{(m-1)(m-2)}(\alpha/2) \times (2S_E^2/m)^{1/2} \quad \ldots (6\cdot49)$$

6·6·2. Least Square Estimates of Parameters. The least square estimates of the $(3m + 1)$ parameters μ, α_i, β_j and τ_k $(i, j, k = 1, 2, \ldots, m)$ in (6·38) are obtained by minimising the residual sum of squares E given by

$$E = \sum_{(i,j,k) \in S} (y_{ijk} - \mu - \alpha_i - \beta_j - \tau_k)^2$$

According to the principle of least squares, the normal equations for estimating μ, α_i, β_j and τ_k are given by :

$$\left.\begin{aligned}
\frac{\partial E}{\partial \mu} &= 0 = \sum_{(i,j,k) \in S} (y_{ijk} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j - \hat{\tau}_k \\
\frac{\partial E}{\partial \alpha_i} &= 0 = \sum_{(j,k) \in S} (y_{ijk} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j - \hat{\tau}_k) \\
\frac{\partial E}{\partial \beta_j} &= 0 = \sum_{(i,k) \in S} (y_{ijk} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j - \hat{\tau}_k) \\
\frac{\partial E}{\partial \tau_k} &= 0 = \sum_{(i,j) \in S} (y_{ijk} - \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j - \hat{\tau}_k)
\end{aligned}\right\} \quad \ldots (*)$$

where S_i is the possible pair (j, k) associated with a fixed value of i and similarly we can define S_j and S_k ; and $\hat{\mu}$, $\hat{\alpha}_i$, $\hat{\beta}_j$, and $\hat{\tau}_k$ are the estimates of μ, α_i , β_j and τ_k respectively.

In order that (*) has a unique solution, we must have

$$\sum_i \hat{\alpha}_i = \sum_j \hat{\beta}_j = \sum_k \hat{\tau}_k = 0 \qquad \ldots (6·50)$$

Since α_i occurs m times for each i, we have from (6·39)

$$\sum_{(i,j,k) \in S} \hat{\alpha}_i = m \sum_i \hat{\alpha}_i = 0 \qquad \ldots (i)$$

Similarly, we have

$$\sum_{(i,j,k) \in S} \hat{\beta}_j = \sum_{(i,j,k) \in S} \hat{\tau}_k = 0 \qquad \ldots (ii)$$

Further, since in an L.S.D. each i and each j occur exactly once with each k in triplet $(i, j, k) \in S$ and consequently S_k consists of m pairs (i, j) and, we get on using (6·40),

$$\left.\begin{aligned}
&\sum_{(i,j) \in S_k} \hat{\alpha}_i = \sum_{(i,k) \in S_j} \hat{\alpha}_i = \sum_i \hat{\alpha}_i = 0 \quad \sum_{(i,j) \in S_k} \hat{\beta}_j = \sum_{(i,k) \in S_i} \hat{\beta}_j = 0 \\
&\text{and} \sum_{(i,j) \in S_k} \hat{\tau}_k = m\hat{\tau}_k, \quad \sum_{(i,k) \in S_i} \hat{\alpha}_i = m\hat{\alpha}_i, \quad \sum_{(i,k) \in S_j} \hat{\beta}_j = m\hat{\beta}_j
\end{aligned}\right\} \quad \ldots (iii)$$

Using (i), (ii) and (iii), we get from (*)

$$\left.\begin{aligned}
y_{...} &= m^2\hat{\mu} \quad \Rightarrow \quad \hat{\mu} = \bar{y}_{...} \; ; \quad y_{i..} = m(\hat{\mu} + \hat{\alpha}_i) \quad \Rightarrow \quad \hat{\alpha}_i = \bar{y}_{i..} - \bar{y}_{...} \\
y_{.j.} &= m(\hat{\mu} + \hat{\beta}_j) \quad \Rightarrow \quad \hat{\beta}_j = \bar{y}_{.j.} - \bar{y}_{...} \; ; \quad y_{..k} = m(\hat{\mu} + \hat{\tau}_k) \quad \Rightarrow \quad \hat{\tau}_k = \bar{y}_{..k} - \bar{y}_{...}
\end{aligned}\right\} \quad \ldots (6·51)$$

6·6·3. Variance of Estimates. From model (6·41), we have

$$\left.\begin{aligned} y_{i..} &= m\mu + m\alpha_i + \sum\beta_j + \sum\tau_k + \varepsilon_{i..} \\ y_{.j.} &= m\mu + \sum\alpha_i + m\beta_j + \sum\tau_k + \varepsilon_{.j.} \\ y_{..k} &= m\mu + \sum\alpha_i + \sum\beta_j + m\tau_k + \varepsilon_{..k} \\ y_{...} &= m^2\mu + m\sum\alpha_i + m\sum\beta_j + m\sum\tau_k + \varepsilon_{...} \end{aligned}\right\} \quad \ldots (6\cdot52)$$

then, from (6·42), we get

$$\left.\begin{aligned} \bar{y}_{i..} &= \mu + \alpha_i + \bar{\varepsilon}_{i..} \;\; ; \;\; \bar{y}_{.j.} = \mu + \beta_j + \bar{\varepsilon}_{.j.} \\ \bar{y}_{..k} &= \mu + \tau_k + \bar{\varepsilon}_{..k} \;\; ; \;\; \bar{y}_{...} = \mu + \bar{\varepsilon}_{...} \end{aligned}\right\} \quad \ldots (6\cdot52a)$$

Since $\varepsilon_{ijk} \sim N(0, \sigma_e^2)$, we get $E(\bar{y}_{...}) = \mu$ ... (6·52*b*)

$$\therefore \quad \text{Var}(\hat{\mu}) = E\left[\hat{\mu} - E(\hat{\mu})\right]^2 = E\left[\bar{y}_{...} - \mu\right]^2 \quad \text{[From (6·51), (6·52}a\text{)] and (6·52}b\text{)]}$$

$$= E(\bar{\varepsilon}_{...})^2 = \text{Var}(\bar{\varepsilon}_{...}) = \frac{\sigma_e^2}{m^2} \quad \ldots (6\cdot53)$$

$$\hat{\alpha}_i = \bar{y}_{i..} - \bar{y}_{...} = \alpha_i + \bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...} \;\Rightarrow\; E(\hat{\alpha}_i) = \alpha_i \quad \text{[Using (6·52}a\text{) and (6·52}b\text{)]}$$

$$\text{Var}(\hat{\alpha}_i) = E[\hat{\alpha}_i - E(\hat{\alpha}_i)]^2 = E(\hat{\alpha}_i - \alpha_i)^2 = E(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2$$

$$= E(\bar{\varepsilon}_{i..}^2) + E(\bar{\varepsilon}_{...}^2) - 2E(\bar{\varepsilon}_{i..}\,\bar{\varepsilon}_{...})$$

$$= \frac{\sigma_e^2}{m} + \frac{\sigma_e^2}{m^2} - 2\left\{\frac{1}{m.m^2}(\sigma_e^2 . m)\right\}, \text{ as in R.B.D.}$$

$$\therefore \quad \text{Var}(\hat{\alpha}_i) = \left(\frac{m-1}{m^2}\right)\sigma_e^2 \quad \ldots (6\cdot53a)$$

Similarly, we shall get

$$\text{Var}(\hat{\beta}_j) = \left(\frac{m-1}{m^2}\right)\sigma_e^2 = \text{Var}(\hat{\tau}_k) \quad \ldots (6\cdot53b)$$

6·6·4. Expectation of Sum of Squares.

$$SSR = m\sum_i(\bar{y}_{i..} - \bar{y}_{...})^2 = m\sum_i(\alpha_i + \bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2$$

$$= m\left[\sum_i \alpha_i^2 + \sum(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2 + 2\sum_i \alpha_i(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})\right]$$

$$\therefore \; E(SSR) = m\left[\sum_i \alpha_i^2 + E\left\{\sum_i(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})^2\right\} + 2\sum_i \alpha_i E(\bar{\varepsilon}_{i..} - \bar{\varepsilon}_{...})\right]$$

$$= m\left[\sum_i \alpha_i^2 + E\left\{\sum_i \bar{\varepsilon}_{i..}^2 - m\,\bar{\varepsilon}_{...}^2\right\} + 0\right] = m\left[\sum_i \alpha_i^2 + \sum_i E(\bar{\varepsilon}_{i..}^2) - m\,E(\bar{\varepsilon}_{...}^2)\right]$$

$$= m\left[\sum_i \alpha_i^2 + \sum_i\left(\frac{\sigma_e^2}{m}\right) - m.\frac{\sigma_e^2}{m^2}\right] = m\left[\sum_i \alpha_i^2 + m.\frac{\sigma_e^2}{m} - \frac{\sigma_e^2}{m}\right]$$

$$= m\sum_i \alpha_i^2 + (m-1)\sigma_e^2$$

$$\Rightarrow E\left(\frac{SSR}{m-1}\right) = \frac{m}{m-1}\sum_i \alpha_i^2 + \sigma_e^2 \Rightarrow E[MSR] = \sigma_e^2 + \frac{m}{m-1}\sum_{i=1}^{m} \alpha_i^2 \quad \ldots (6\text{-}54)$$

(MSR = Mean S.S. due to rows = s_E^2)

Similarly, we can show that

$$E(SSC) = m\sum_j \beta_j^2 + (m-1)\sigma_e^2$$

$$\Rightarrow E(MSC) = E\left(\frac{SSC}{m-1}\right) = \sigma_e^2 + \frac{m}{m-1}\sum_{j=1}^{m} \beta_j^2 \quad \ldots (6\text{-}55)$$

and $$E(SST) = m\sum_k \tau_k^2 + (m-1)\sigma_e^2$$

$$\Rightarrow E(MST) = E\left(\frac{SST}{m-1}\right) = \sigma_e^2 + \frac{m}{m-1}\sum_{k=1}^{m} \tau_k^2 \quad \ldots (6\text{-}56)$$

$$SSE = \sum_{i,\, j,\, k \in S} \left(y_{ijk} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} - \bar{y}_{\cdot\cdot k} + 2\bar{y}_{\cdots}\right)^2$$

$$= \sum_{i,\, j,\, k \in S} \left(\varepsilon_{ijk} - \bar{\varepsilon}_{i\cdot\cdot} - \bar{\varepsilon}_{\cdot j\cdot} - \bar{\varepsilon}_{\cdot\cdot k} + 2\bar{\varepsilon}_{\cdots}\right)^2 \quad \text{[On using (6-52a)]}$$

$$= \sum_{i,\, j,\, k \in S} \left[\varepsilon_{ijk}^2 + \bar{\varepsilon}_{i\cdot\cdot}^2 + \bar{\varepsilon}_{\cdot j\cdot}^2 + \bar{\varepsilon}_{\cdot\cdot k}^2 + 4\bar{\varepsilon}_{\cdots}^2\right]$$

$$+ 2\left\{-\varepsilon_{ijk}\bar{\varepsilon}_{i\cdot\cdot} - \varepsilon_{ijk}\bar{\varepsilon}_{\cdot j\cdot} - \varepsilon_{ijk}\bar{\varepsilon}_{\cdot\cdot k} + 2\varepsilon_{ijk}\bar{\varepsilon}_{\cdots}\right.$$

$$\left. + \bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdot j\cdot} + \bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdot\cdot k} + \bar{\varepsilon}_{\cdot j\cdot}\bar{\varepsilon}_{\cdot\cdot k} - 2\bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdots} - 2\bar{\varepsilon}_{\cdot j\cdot}\bar{\varepsilon}_{\cdots} - 2\bar{\varepsilon}_{\cdot\cdot k}\bar{\varepsilon}_{\cdots}\right\}\Big]$$

We have

$$E(\varepsilon_{ijk}^2) = \sigma_e^2;\ E(\bar{\varepsilon}_{i\cdot\cdot}^2) = (\bar{\varepsilon}_{\cdot j\cdot}^2) = E(\bar{\varepsilon}_{\cdot\cdot k}^2) = \frac{\sigma_e^2}{m} \text{ and } E(\bar{\varepsilon}_{\cdots})^2 = \frac{\sigma_e^2}{m^2} \quad \ldots (6\text{-}57)$$

$$E(\varepsilon_{ijk}\bar{\varepsilon}_{i\cdot\cdot}) = E\left[\varepsilon_{ijk}\left(\frac{\sum_{j,k \in S}\varepsilon_{ijk}}{m}\right)\right] = \frac{1}{m}E(\varepsilon_{ijk}^2) = \frac{\sigma_e^2}{m} \quad (\because \varepsilon_{ijk}\text{'s are independent}) \quad \ldots (6\text{-}57a)$$

Similarly

$$E(\varepsilon_{ijk}\bar{\varepsilon}_{\cdot j\cdot}) = E(\varepsilon_{ijk}\bar{\varepsilon}_{\cdot\cdot k}) = \frac{\sigma_e^2}{m} \quad \ldots (6\text{-}57b)$$

$$E(\varepsilon_{ijk}\bar{\varepsilon}_{\cdots}) = E\left[\varepsilon_{ijk}\left(\frac{\sum_{i,j,k}\varepsilon_{ijk}}{m^2}\right) = \frac{1}{m^2}E(\varepsilon_{ijk}^2) = \frac{\sigma_e^2}{m^2}\right. \quad \ldots (6\text{-}57c)$$

$$E(\bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdot j\cdot}) = E\left[\left(\frac{\sum\varepsilon_{ijk}}{\underset{j,k}{m}}\right)\left(\frac{\sum\varepsilon_{ijk}}{\underset{i,k}{m}}\right)\right] = \frac{1}{m^2}E(\varepsilon_{ijk}^2) = \frac{\sigma_e^2}{m^2} \quad \ldots (6\text{-}57d)$$

$$E(\bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdot\cdot k}) = E(\bar{\varepsilon}_{\cdot j\cdot}\bar{\varepsilon}_{\cdot\cdot k}) = \frac{\sigma_e^2}{m^2} \quad \ldots (6\text{-}57e)$$

$$E(\bar{\varepsilon}_{i\cdot\cdot}\bar{\varepsilon}_{\cdots}) = E\left[\frac{1}{m}\left(\sum_{j,k \in S}\varepsilon_{ijk}\right)\cdot\frac{1}{m^2}\left(\sum_{i,j,k \in S}\varepsilon_{ijk}\right)\right] = \frac{1}{m^3}\left[m\,E(\varepsilon_{ijk}^2)\right] = \frac{\sigma_e^2}{m^2} \quad \ldots (6\text{-}57f)$$

($\because$ The two sums have m elements common, corresponding to the ith row)

Similarly,

$$E(\bar{\varepsilon}_{.j.}\,\bar{\varepsilon}_{...}) = E(\bar{\varepsilon}_{..k}\,\bar{\varepsilon}_{...}) = \frac{\sigma_e^2}{m^2} \qquad \ldots (6\cdot57g)$$

Taking expectations of both sides in (6·56*a*) and substituting from (6·57) to (6·57*g*), we get :

$$E(SSE) = \sum_{i,j,k \in S} \left[\sigma_e^2 \left\{1 + \frac{1}{m} + \frac{1}{m} + \frac{1}{m} + 4\frac{1}{m^2} + 2\left(-\frac{1}{m} - \frac{1}{m} - \frac{1}{m} + \frac{2}{m^2} + \frac{1}{m^2} + \frac{1}{m^2} + \frac{1}{m^2} - \frac{2}{m^2} - \frac{2}{m^2} - \frac{2}{m^2}\right)\right\}\right]$$

$$= m^2\,\sigma_e^2\left(1 + \frac{3}{m} - \frac{6}{m} + \frac{4}{m^2} - \frac{2}{m^2}\right) = \sigma_e^2\,(m^2 - 3m + 2)$$

$$= (m-1)(m-2)\,\sigma_e^2$$

$$\Rightarrow \qquad E(MSE) = E\left[\frac{SSE}{(m-1)(m-2)}\right] = \sigma_e^2 \qquad \ldots (6\cdot58)$$

$\Rightarrow$ **Error mean sum of squares (MSE = s_E^2) provides an unbiased estimate of the population variance σ_e^2.** *Under the null hypotheses :*

$H_{0\alpha} : \alpha_1 = \alpha_2 = \ldots = \alpha_m = 0$; $H_{0\beta} : \beta_1 = \beta_2 = \ldots = \beta_m = 0$ and $H_{0\tau} : \tau_1 = \tau_2 = \ldots = \tau_m = 0$ $\qquad \ldots (6\cdot59)$

We get from (6·54), (6·55) and (6·56) respectively :

$$E\left(\frac{SSR}{m-1}\right) = E(MSR) = \sigma_e^2 \;;\; E\left(\frac{SSC}{m-1}\right) = E(MSC) = \sigma_e^2 \;;\; E\left(\frac{SST}{m-1}\right) = E(MST) = \sigma_e^2 \qquad \ldots (6\cdot59a)$$

i.e., each of the mean sum of squares due to rows, columns and treatments gives an unbiased estimate σ_e^2 under the null hypotheses $H_{0\alpha}$ $H_{0\beta}$ and $H_{0\tau}$ respectively.

6·6·5. Latin Square Design—Random Effect Model. The linear model (for random effects) in *LSD* is given by :

$$y_{ijk} = \mu + r_i + c_j + t_k + \varepsilon_{ijk}\,, \qquad \ldots (6\cdot60)$$

where

μ is the general mean effect,

r_i is the additional effect due to ith row

c_j is the additional effect due to jth column

t_k is the additional effect due to kth treatment $(i, j, k = 1, 2, \ldots, m)$

and

$$\left.\begin{aligned} & r_i \overset{i.i.d}{\sim} N(0, \sigma_r^2)\,,\ i = 1, 2, \ldots, m \\ & c_j \overset{i.i.d}{\sim} N(0, \sigma_c^2),\ j = 1, 2, \ldots, m \\ & t_k \overset{i.i.d}{\sim} N(0, \sigma_t^2),\ k = 1, 2, \ldots, m \\ & \varepsilon_{ijk} \overset{i.i.d}{\sim} N(0, \sigma_e^2)\ \forall\ i, j, k = 1, 2, \ldots, m \end{aligned}\right\} \qquad \ldots (6\cdot60a)$$

and r_i, c_j, t_k and ε_{ijk} are distributed independent of each other, so that

$$\left.\begin{aligned} &E(r_i) = E(c_j) = E(t_k) = 0\ ;\ i, j, k = 1, 2, \ldots, m \\ &\text{Var}\,(r_i) = E(r_i^2) = \sigma_r^2\ ;\ \text{Var}\,(c_j) = E(c_j^2) = \sigma_c^2 \\ &\text{Var}\,(t_k) = E(t_k^2) = \sigma_t^2\ ;\ \text{Var}\,(\varepsilon_{ijk}) = E(\varepsilon_{ijk}^2) = \sigma_e^2 \\ &\text{Cov}\,(r_i, c_j) = \text{Cov}\,(r_i, t_k) = \text{Cov}\,(c_j, t_k) = 0 \\ &\text{Cov}\,(r_i, \varepsilon_{ijk}) = \text{Cov}\,(c_j, \varepsilon_{ijk}) = \text{Cov}\,(t_k, \varepsilon_{ijk}) = 0 \end{aligned}\right\} \quad \ldots (6{\cdot}60b)$$

From (6·60), we get

$$\left.\begin{aligned} y_{i\cdot\cdot} &= m\mu + mr_i + \sum_j c_j + \sum_k t_k + \varepsilon_{i\cdot\cdot} \\ y_{\cdot j\cdot} &= m\mu + \sum_i r_i + mc_j + \sum_k t_k + \varepsilon_{\cdot j\cdot} \\ y_{\cdot\cdot k} &= m\mu + \sum_i r_i + \sum_j c_j + mt_k + \varepsilon_{\cdot\cdot k} \\ y_{\ldots} &= m^2\mu + m\sum r_i + m\sum c_j + m\sum t_k + \varepsilon_{\ldots} \end{aligned}\right\} \quad \ldots (6{\cdot}61)$$

$$\therefore \quad E(y_{ijk}^2) = E(\mu + r_i + c_j + t_k + \varepsilon_{ijk})^2 = \mu^2 + \sigma_r^2 + \sigma_c^2 + \sigma_t^2 + \sigma_e^2 \quad \text{[Using (6·60}b\text{)]} \quad \ldots (6{\cdot}62)$$

$$E(y_{i\cdot\cdot}^2) = E(m\mu + mr_i + \sum_j c_j + \sum_k t_k + \varepsilon_{i\cdot\cdot})^2$$

$$= m^2\mu^2 + m^2 E(r_i^2) + \sum_j E(c_j^2) + \sum_k E(t_k^2) + E(\varepsilon_{i\cdot\cdot}^2)$$

[All covariance terms vanish, because of (6·60b)]

$$\Rightarrow \quad E(y_{i\cdot\cdot}^2) = m^2\mu^2 + m^2\sigma_r^2 + m\sigma_c^2 + m\,\sigma_t^2 + m\sigma_e^2$$

($\because$ $\varepsilon_{i\cdot\cdot}^2$ is the sum of squares of m *i.i.d.* observations)

$$= m^2(\mu^2 + \sigma_r^2) + m(\sigma_c^2 + \sigma_t^2 + \sigma_e^2) \quad \ldots (6{\cdot}62a)$$

Similarly from (6·61), on using (6·60b), we shall get

$$E(y_{\cdot j\cdot}^2) = m^2(\mu^2 + \sigma_c^2) + m(\sigma_r^2 + \sigma_t^2 + \sigma_e^2) \quad \ldots (6{\cdot}62b)$$

$$E(y_{\cdot\cdot k}^2) = m^2(\mu^2 + \sigma_t^2) + m(\sigma_r^2 + \sigma_t^2 + \sigma_e^2) \quad \ldots (6{\cdot}62c)$$

$$E(y_{\ldots}^2) = E\left[m^2\mu + m\sum_i r_i + m\sum_i c_j + m\sum_k t_k + \varepsilon_{\ldots}\right]^2$$

$$= \left[m^4\mu^2 + m^2 m\sigma_r^2 + m^2 m\sigma^2 + m^2 m\sigma_t^2 + m^2\sigma_e^2\right]$$

$$= m^2\left[m^2\mu^2 + m(\sigma_r^2 + \sigma_c^2 + \sigma_t^2) + \sigma_e^2\right] \quad \ldots (6{\cdot}62d)$$

$$\therefore E(SSR) = E\left[\sum_i (\bar{y}_{i\cdot\cdot} - \bar{y}_{\ldots})^2\right] = E\left[\frac{1}{m}\sum_i y_{i\cdot\cdot}^2 - C.F.\right]$$

$$= E\left[\frac{1}{m}\sum_i y_{i\cdot\cdot}^2 - \frac{y_{\ldots}^2}{m^2}\right] = \frac{1}{m}\sum_i E(y_{i\cdot\cdot}^2) - \frac{1}{m^2}E(y_{\ldots}^2)$$

$$= \frac{1}{m}\left[\sum_i \left\{m^2(\mu^2 + \sigma_r^2) + m(\sigma_c^2 + \sigma_t^2 + \sigma_e^2)\right\}\right] - \frac{1}{m^2}\left[m^2\left\{m^2\mu^2 + m(\sigma_r^2 + \sigma_c^2 + \sigma_t^2) + \sigma_e^2\right\}\right]$$

[From (6·62a) and (6·62d)]

$$= m^2(\mu^2 + \sigma_r^2) + m(\sigma_c^2 + \sigma_t^2 + \sigma_e^2) - \left[m^2\mu^2 + m(\sigma_r^2 + \sigma_c^2 + \sigma_t^2) + \sigma_e^2\right]$$

$$= (m^2 - m)\,\sigma_r^2 + (m-1)\,\sigma_e^2 = (m-1)\left[m\,\sigma_r^2 + \sigma_e^2\right]$$

$$\therefore \quad E\left(\frac{SSR}{m-1}\right) = m\sigma_r^2 + \sigma_e^2 \quad \Rightarrow \quad E(MSR) = E\left(s_R^2\right) = \sigma_e^2 + m\sigma_r^2 \qquad \ldots (6\cdot63)$$

Similarly, we can prove that

$$E(SSC) = (m-1)(\sigma_e^2 + m\,\sigma_c^2) \quad \Rightarrow \quad E\left[\frac{SSC}{m-1}\right] = \sigma_e^2 + m\,\sigma_c^2$$

$$\Rightarrow \quad E(MSC) = E\left(s_c^2\right) = \sigma_e^2 + m\,\sigma_c^2 \qquad \ldots (6\cdot64)$$

and

$$E(MST) = E\left(s_t^2\right) = \sigma_e^2 + m\,\sigma_t^2 \qquad \ldots (6\cdot65)$$

$$E(SSE) = E\,[\,TSS - SSR - SSC - SST\,]$$

$$= E\left[\sum_{i,j,k\varepsilon S} y_{ijk}^2 - \frac{1}{m}\sum_i y_{i\cdot\cdot}^2 - \frac{1}{m}\sum_i y_{\cdot j\cdot}^2 - \frac{1}{m}\sum_k y_{\cdot\cdot k}^2 + 2\frac{y_{\ldots}^2}{m^2}\right]$$

[*c.f.* Remark 1 to § 6·7·1, equations (6·43) to (6·47)]

$$= m^2\left(\mu^2 + \sigma_r^2 + \sigma_c^2 + \sigma_t^2 + \sigma_e^2\right) = -\frac{1}{m}\cdot m\left\{m^2(\mu^2 + \sigma_r^2) + m(\sigma_c^2 + \sigma_t^2 + \sigma_e^2)\right\}$$

$$-\frac{1}{m}\cdot m\left\{m^2(\mu^2 + \sigma_c^2) + m(\sigma_r^2 + \sigma_t^2 + \sigma_e^2)\right\} - \frac{1}{m}\cdot m\left\{m^2(\mu^2 + \sigma_t^2) + m(\sigma_r^2 + \sigma_c^2 + \sigma_e^2)\right\}$$

$$+ \frac{2}{m^2}\cdot m^2\left\{m^2\mu^2 + m(\sigma_r^2 + \sigma_c^2 + \sigma_t^2) + \sigma_e^2\right\} \qquad [\text{From } (6\cdot62a) \text{ to } (6\cdot62d)]$$

$$= \sigma_e^2(m^2 - m - m - m + 2) = (m^2 - 3m + 2)\,\sigma_e^2 = (m-1)(m-2)\,\sigma_e^2$$

$$\therefore \quad E\left[\frac{SSE}{(m-1)(m-2)}\right] = \sigma_e^2 \quad \Rightarrow \quad E(MSE) = E\left(s_E^2\right) = \sigma_e^2 \qquad \ldots (6\cdot66)$$

Hence, MSE provides an unbiased estimate of σ_e^2.

$$\therefore \quad \hat{\sigma}_e^2 = MSE \qquad \ldots (6\cdot66a)$$

Estimates of σ_r^2, σ_c^2 **and** σ_t^2. From (6·63), we get

$$E(MSR) = \sigma_e^2 + m\sigma_r^2 \quad \Rightarrow \quad \left(\sigma_e^2 + m\,\sigma_r^2\right) = MSR$$

$$\Rightarrow \quad \hat{\sigma}_e^2 + m\,\hat{\sigma}_r^2 = MSR \quad \Rightarrow \quad \hat{\sigma}_r^2 = \frac{MSR - MSE}{m} \qquad [\text{From } (6\cdot66a)] \qquad \ldots (6\cdot67)$$

Similarly, $E(MSC) = \sigma_e^2 + m\,\sigma_c^2$ [From (6·64)]

$$\Rightarrow \quad (\hat{\sigma}_e^2 + m\,\sigma_c^2) = MSC \quad \Rightarrow \quad \hat{\sigma}_c^2 = \frac{MSC - MSE}{m} \qquad \ldots (6\cdot68)$$

$$E(MST) = \sigma_e^2 + m\,\sigma_t^2 \text{ [From (6·65)]} \quad \Rightarrow \quad \hat{\sigma}_e^2 = \frac{MST - MSE}{m} \qquad \ldots (6\cdot69)$$

Null Hypothesis. The appropriate hypothesis for testing the equality of means due to different factors, viz., rows, columns and treatments are :

$$\left.\begin{array}{lllll} (i)\ H_{or}: & \sigma_r^2 = 0 & \text{against} & H_{1r}: \sigma_r^2 > 0 \\ (ii)\ H_{oc}: & \sigma_c^2 = 0 & \text{against} & H_{1c}: \sigma_e^2 > 0 \\ (iii)\ H_{ot}: & \sigma_t^2 = 0 & \text{against} & H_{1t}: \sigma_t^2 > 0 \end{array}\right\} \quad \ldots (6\cdot70)$$

Under these null hypotheses, we get from (6·63), (6·64) and (6·65) respectively :

$$E(MSR) = \sigma_e^2 \ ; \ E(MSC) = \sigma_e^2 \text{ and } E(MST) = \sigma_e^2$$

i.e., Each of the mean sum of squares due to rows, columns and treatments gives an unbiased estimate of σ_e^2 under the null hypotheses : H_{or}, H_{oc} and H_{ot} respectively whereas MSE always gives unbiased estimate of σ_e^2.

Hence, as in the fixed effect model, the test statistics for testing H_{or}, H_{oc} and H_{ot} are given respectively by :

$$\left.\begin{array}{l} F_R = \dfrac{MSR}{MSE} \sim F_{m-1,\,(m-1)(m-2)} \\ F_c = \dfrac{MSC}{MSE} \sim F_{m-1,\,(m-1)(m-2)} \\ \text{and} \quad F_t = \dfrac{MST}{MSE} \sim F_{m-1,\,(m-1)(m-2)} \end{array}\right\} \quad \ldots (6\cdot71)$$

Hence, the ANOVA table for LSD for *Random Effect Model* is similar to that of ANOVA table for *fixed effects*.

Remark. From the above discussion we observe that the computational procedure for finding the various sum of squares and the corresponding statistical analysis of the Random Effect Model is exactly similar to that of the fixed effect model of LSD. However, the conclusions drawn from the analysis of these models are quite different. In the fixed effect model, the conclusions drawn are valid *only* for the set of treatments used in the experiment. On the other hand, in the random effect model, the conclusions drawn are valid for the entire class of treatments from which the given set of treatments used in the experiment, is drawn as a random sample.

6·6·6. Efficiency of LSD Relative to RBD. ANOVA Table for Latin Square Design (LSD) with m treatments for Random Effects [*c.f.* (6·63) to (6·65)] is given in *Table 6·20*.

TABLE 6·20 : ANOVA TABLE (LSD) (RANDOM EFFECT MODEL)

Sources of Variation	*d.f.*	*Mean S.S.*	*E(MSS)*
Rows	$m-1$	s_R^2	$\sigma_e^2 + m\sigma_r^2$
Columns	$m-1$	s_C^2	$\sigma_e^2 + m\,\sigma_c^2$
Treatments	$m-1$	s_t^2	$\sigma_e^2 + m\,\sigma_t^2$
Error	$(m-1)(m-2)$	s_E^2	σ_e^2
Total	m^2-1	s_T^2	

Efficiency of LSD Relative to RBD.

Case 1. *Rows of LSD as Blocks.* Let $s_{E'}^2$ be the error mean sum of squares for *RBD* with rows (of *LSD*) as blocks. Then the efficiency of *LSD* relative to *RBD* is given by :

$$E_1 = \frac{s_{E'}^2}{s_E^2} \quad \ldots (6\cdot72)$$

If we apply uniformity trials to LSD which consists in using the same treatment on all the m^2 units, there are no treatment variations. Consequently the treatment *d.f.* add to the error *d.f.* Therefore

Error *d.f.* (due to uniformity trials) $= (m-1)(m-2) + (m-1) = (m-1)[m-2+1] = (m-1)^2$ ... (6·73)

$\Rightarrow$ Error *S.S.* $= (d.f.) \times$ Mean *S.S.* $= (m-1)^2 s_E^2$ ... (6·74)

Now, if we conduct the same experiment as RBD with rows as blocks, there are no variations due to columns. Hence, the columns *d.f.* and column *s.s* (add to the error *d.f.* and error *s.s.*) respectively.

Hence, for *RBD*

Error *d.f.* $= (m-1)^2 + (m-1) = (m-1)(m-1+1) = m(m-1)$ [From (6·73)]

and Error S.S. $= (m-1)^2 s_E^2 + (m-1) s_c^2$ [From (6·74)]

$\therefore$ Error mean square for *RBD* (with Rows as Blocks) is

$$s_{E'}^2 = \frac{(m-1)^2 s_E^2 + (m-1) s_c^2}{m(m-1)} = \frac{(m-1) s_E^2 + s_c^2}{m} \quad \text{... (6·75)}$$

Hence, the efficiency E_1 of *LSD* relative to *RBD* (with rows as blocks) is given by :

$$E_1 = \frac{s_{E'}^2}{s_E^2} = \frac{s_c^2 + (m-1) s_E^2}{m s_E^2} \quad \text{... (6·76)}$$

we can re-write :

$$E_1 = \frac{m s_E^2 + (s_c^2 - s_E^2)}{m s_E^2} = 1 + \left(\frac{s_c^2 - s_E^2}{m}\right) \cdot \frac{1}{s_E^2} \quad \text{... (6·76a)}$$

From the ANOVA *Table 6·20*, we observe that

$$E\left(s_c^2 - s_E^2\right) = E\left(s_c^2\right) - E\left(s_E^2\right) = \sigma_e^2 + m\sigma_c^2 - \sigma_e^2 = m\sigma_c^2 \quad \Rightarrow \quad \text{Est}\left(\frac{s_c^2 - s_E^2}{m}\right) = \sigma_e^2$$

Substituting in (6·76*a*), we get

$$E_1 = 1 + \frac{\sigma_c^2}{s_E^2} \quad \Rightarrow \quad E_1 \geq 1. \quad \text{... (6·76b)}$$

$E_1 = 1$ if $\sigma_c = 0$ i.e., both LSD and RBD (with rows as blocks) are equally efficient if $\sigma_c = 0$, otherwise ($\sigma_c \neq 0$), LSD is more efficient than RBD.

Case 2. *Column of LSD as Blocks.* As in case (1) as a result of uniformity trials in LSD,

$$\left.\begin{aligned} &\text{Error } d.f. = (m-1)^2 \\ \text{and} \quad &\text{Error } S.S. = (m-1)^2 s_E^2 \end{aligned}\right\} \quad (*)$$

If we conduct the same experiment as *RBD* with columns of *LSD* as blocks, there will be no variations due to rows. Hence the row *d.f.* and row *S.S.* for *LSD* add to the error *d.f.* and error *S.S.* respectively

Hence *for RBD with (columns as blocks)*,

$$\left.\begin{aligned} &\text{Error } d.f. = (m-1)^2 + (m-1) = m(m-1) \\ \text{and} \quad &\text{Error } S.S. = (m-1)^2 s_E^2 + (m-1) s_k^2 \end{aligned}\right\} \quad \text{(From (*))}$$

$\therefore$ Error mean square for *RBD* (columns as blocks) is :

$$s_{E''}^2 = \frac{(m-1)^2 s_{E''}^2 + (m-1) s_R^2}{m(m-1)} = \frac{s_R^2 + (m-1) s_E^2}{m}$$

∴ Efficiency E_2 of *LSD* relative to *RBD* (with columns as blocks) is given by :

$$E_2 = \frac{s_{E''}^2}{s_E^2} = \frac{s_R^2 + (m-1)\,s_E^2}{m\,s_E^2} \quad \ldots (6\cdot77)$$

Rewriting, we have

$$E_2 = \frac{s_R^2 + (m-1)\,s_E^2}{m\,s_E^2} = 1 + \left(\frac{s_R^2 - s_E^2}{m}\right)\frac{1}{s_E^2}$$

As in case (1), from *Table 6·20*, we shall get

$$E\left(s_R^2 - s_E^2\right) = m\,\sigma_r^2 \quad \Rightarrow \quad \left(\frac{s_R^2 - s_E^2}{m}\right) = \sigma_r^2 \quad \ldots (6\cdot77a)$$

$$\therefore \quad E_2 = 1 + \frac{\sigma_r^2}{s_E^2} \quad \Rightarrow \quad E_2 \geq 1$$

Thus, $E_2 = 1$ if $\sigma_r = 0$ *i.e.*, *LSD* is equally efficient as *RBD* (with columns as blocks) if $\sigma_r = 0$, otherwise *LSD* is more efficient than *RBD*.

6·6·7. Efficiency of LSD Relative of CRD. As in the above cases (1) and (2) in § 6·7·6., first of all, we apply uniformity trials to LSD so that

$$\left.\begin{aligned} \text{Error } d.f. &= (m-1)^2 \\ \text{Error } S.S. &= (m-1)^2\,s_E^2 \end{aligned}\right\} \quad \ldots (6\cdot78)$$

Now, if we conduct the same LSD experiment as a *CRD* experiment, there is no local control. Consequently the *d.f.* and the *S.S.* for blocks and rows will add to the *d.f.* and *S.S.* for error respectively. Thus, for *CRD*.

$$\text{Error } d.f. = (m-1)^2 + (m-1) + (m-1) = m^2 - 1 \quad \text{[From 6·78)]}$$

and $$\text{Error } S.S. = (m-1)^2\,s_E^2 + (m-1)\,s_R^2 + (m-1)\,s_c^2$$

∴ Error mean square $\left(s_{E'''}^2\right)$ for *CRD* is given by :

$$s_{E'''}^2 = \frac{\text{Error } S.S.}{\text{Error } d.f.} = \frac{(m-1)^2\,s_E^2 + (m-1)\,s_R^2 + (m-1)\,s_c^2}{m^2-1} = \frac{s_R^2 + s_c^2 + (m-1)\,s_E^2}{(m+1)}$$

Hence, the efficiency E_3 of *LSD* relative to *CRD* is given by :

$$E_3 = \frac{s_{E'''}^2}{s_E^2} = \frac{s_R^2 + s_c^2 + (m-1)\,s_E^2}{(m+1)\,s_E^2} \quad \ldots (6\cdot79)$$

Re-writing, we have

$$E_3 = \frac{s_R^2 + s_c^2 + (m-1)\,s_E^2}{(m-1)\,s_E^2} = \frac{s_R^2 + s_c^2 + (m+1-2)\,s_E^2}{(m-1)\,s_E^2}$$

$$= 1 + \frac{s_R^2 + s_c^2 - 2s_E^2}{(m+1)\,s_E^2} = 1 + \frac{\left(s_R^2 - s_E^2\right) + \left(s_c^2 - s_E^2\right)}{(m+1)\,s_E^2}$$

But, from *Table 6·20*, we have

$$E\left(s_R^2 - s_E^2\right) = m\sigma_r^2 \quad \Rightarrow \quad \left(s_R^2 - s_E^2\right) = m\sigma_r^2$$

and $$E\left(s_c^2 - s_E^2\right) = m\sigma_c^2 \quad \Rightarrow \quad \left(s_c^2 - s_E^2\right) = m\sigma_c^2$$

$$\therefore \quad E_3 = 1 + \frac{m(\sigma_r^2 + \sigma_c^2)}{(m+1)\,s_E^2} \geq 0 \qquad \ldots (6\cdot79\alpha)$$

Thus, $E_3 = 1$, if and only if $\sigma_r = 0$ and $\sigma_c = 0$ *i.e.*, *LSD* is equally efficient as *CRD* if and only if $\sigma_r = 0$ and $\sigma_c = 0$, otherwise *LSD* is more efficient than *CRD*.

Remarks **1.** As stated in working out the efficiency of *RBD* relative to *CRD* [See Remark 1 to § 6·6·6, Aliter Method], the results in (6·76), (6·77) and (6·79) are based on the basic assumption that exactly the same experimental units are used in either of the *RBD* and *CRD* as in *LSD*.

2. *Some results on Efficiencies.* ***Cochran W.G.,*** on the basis of a series of field experiments conducted at Rothamsted and associated centres during the years 1927 to 1934 obtained the following results for the efficiencies of *LSD* relative to *RBD* and *CRD*.

Efficiency of *LSD* relative to *RBD* = 137%

Efficiency of *LSD* relative to *CRD* = 222%

Now 100 : 137 : 222 : : 4·50 : 6·17 : 10

This implies that approximately 10 replicates of a *CRD* are equivalent to 6 replicates of a *RBD* and 4 or 5 replicates of an *LSD*, to achieve a particular accuracy.

These figures may be regarded as an indicator of the type of results that may be obtained with different experimental material.

6·6·8. Estimation of Missing Values in Latin Square Design. Let us suppose that in $m \times m$ Latin Square, the observation occurring in the ith row, jth column and receiving the kth treatment is missing. Let us assume that its value is x, *i.e.*, $y_{ijk} = x$.

R = Total of the *known* observations in the ith row, *i.e.*, the row containing 'x'.

C = Total of known observations in the jth column, *i.e.*, the column containing 'x'.

T = Total of known observations receiving kth treatment, *i.e.*, total of all known treatment values containing 'x'.

S = Total of known observations.

Then

$$\text{S.S.R.} = \frac{(R+x)^2}{m} + \text{constant } w.r.t.\ x - \frac{(S+x)^2}{m^2}$$

$$\text{S.S.C.} = \frac{(C+x)^2}{m} + \text{constant } w.r.t.\ x - \frac{(S+x)^2}{m^2}$$

$$\text{S.S.T.} = \frac{(T+x)^2}{m} + \text{constant } w.r.t.\ x - \frac{(S+x)^2}{m^2}$$

$$\therefore \quad E = \text{Residual Sum of Squares (S.S.E.)} = \text{T.S.S.} - \text{S.S.R.} - \text{S.S.C.} - \text{S.S.T.}$$

$$= x^2 - \frac{1}{m}\left[(R+x)^2 + (C+x)^2 + (T+x)^2\right] + 2\,\frac{(S+x)^2}{m^2}$$

We will choose x so as to minimise E.

$$\therefore \quad \frac{\partial E}{\partial x} = 0 = 2x - \frac{2}{m}(R + C + T + 3x) + \frac{4(S+x)}{m^2}$$

$$\Rightarrow \quad (m^2 - 3m + 2)\,x = m(R + C + T) - 2S$$

$$\Rightarrow \quad \hat{x} = \frac{m(R + C + T) - 2S}{(m-1)(m-2)} \qquad \ldots (6\cdot80)$$

Remark. The same procedure may be followed for estimating more than one, say k missing values and then missing values are obtained by solving k-equations simultaneously.

Statistical Analysis. After inserting the estimated value for missing observation, we perform the usual analysis of variance, subtracting one *d.f.* for total S.S. and consequently for Error S.S. Adjusted treatment S.S. is obtained by subtracting the quantity

$$\left[(m-1)\,T + R + C - S\right]^2 / \left[(m-1)\,(m-2)\right]^2 \qquad \ldots (6{\cdot}80a)$$

from the treatment S.S.

Standard Error (*S.E.*) of the difference between two treatment means, none of which corresponds to missing values is given by $S_E\sqrt{2/m}$. The S.E. of difference of the means of two treatments, one of which corresponds to missing observation is given by :

$$s_E\left[\frac{2}{m} + \frac{1}{(m-1)\,(m-2)}\right]^{\frac{1}{2}} \qquad \ldots (6{\cdot}81)$$

provided the treatments show significant effect.

For a detailed discussion on the estimation of missing observation in LSD and its statistical analysis, see § 6·9 in *Missing Plot Technique.*

Example 6·7. *An experiment was carried out to determine the effect of claying the ground on the field of barley grains; amount of clay used were as follows :*

A : No clay
B : Clay at 100 per acre
C : Clay at 200 per acre
D : Clay at 300 per acre.

The yields were in plots of 8 metres by 8 metres and are given in Table 6·21.

TABLE 6·21

Column → / Row ↓	*I*	*II*	*III*	*IV*	*Row totals* (R_i)
I	*D* 29·1	*B* 18·9	*C* 29·4	*A* 5·7	83·1
II	*C* 16·4	*A* 10·2	*D* 21·2	*B* 19·1	66·9
III	*A* 5·4	*D* 38·8	*B* 24·0	*C* 37·0	105·2
IV	*B* 24·9	*C* 41·7	*A* 9·5	*D* 28·9	105·0
Column Totals (C_j)	75·8	109·6	84·1	90·7	360·2

(a) *Perform the ANOVA and calculate the critical difference for the treatment mean yields.*

(b) *Calculate the efficiency of the above Latin Square Design over (i) R.B.D. and (ii) C.R.D.*

(c) *Yield under 'A' in the first column was missing. Estimate the missing value and carry out the ANOVA.*

Solution. The four treatment *totals* are :

A : 30·8, B : 86·9, C : 124·5, D : 118·0

Grand total $G = 360{\cdot}2$, $N = 16$.

$$\text{C.F.} = \frac{(360{\cdot}2)^2}{16} = 8109{\cdot}0025$$

$$\text{Raw S.S.} = (29{\cdot}1)^2 + (18{\cdot}9)^2 + \ldots + (9{\cdot}5)^2 + (28{\cdot}9)^2 = 10{,}052{\cdot}08$$

$\therefore$ Total S.S. $= 10{,}052{\cdot}08 - 8{,}109{\cdot}0025 = 1{,}943{\cdot}0775$

$$\text{S.S.R.} = \frac{1}{4}\left[(83{\cdot}1)^2 + (66{\cdot}9)^2 + (105{\cdot}2)^2 + (105{\cdot}0)^2\right] - 8{,}109{\cdot}0025$$

$$= \frac{33{,}473{\cdot}26}{4} - 8{,}109{\cdot}0025 = 259{\cdot}3125$$

$$\text{S.S.C.} = \frac{1}{4}\left[(75{\cdot}8)^2 + (109{\cdot}6)^2 + (84{\cdot}1)^2 + (90{\cdot}7)^2\right] - 8{,}109{\cdot}0025$$

$$= \frac{33057{\cdot}10}{4} - 8109{\cdot}0025 = 155{\cdot}2725$$

$$\text{S.S.T.} = \frac{1}{4}\left[(30{\cdot}8)^2 + (86{\cdot}9)^2 + (124{\cdot}5)^2 + (118{\cdot}0)^2\right] - 8{,}109{\cdot}0025$$

$$= \frac{37924{\cdot}50}{4} - 8109{\cdot}0025 = 1372{\cdot}1225$$

Error S.S. = T.S.S. – S.S.R. – S.S.C. – S.S.T. = 156·3700

TABLE 6·22 : ANOVA TABLE FOR L.S.D.

Source of variation	*d.f.*	*S.S.*	*M.S.S.*	*Variance Ratio*
(1)	(2)	(3)	(4) = (3) ÷ (2)	
Rows	3	259·5375	86·4375	$F_R = \frac{86{\cdot}4375}{26{\cdot}0616} = 3{\cdot}32 < 4{\cdot}76$
Columns	3	155·2725	51·7575	$F_c = \frac{51{\cdot}7575}{26{\cdot}0616} = 1{\cdot}98 < 4{\cdot}76$
Treatments	3	1,372·1225	457·3742	$F_T = \frac{457{\cdot}3742}{26{\cdot}0616} = 17{\cdot}55 > 4{\cdot}76$
Error	6	156·3700	26·0616	
Total	15	1,943·0775		

Tabulated $F_{3,6}(0{\cdot}05) = 4{\cdot}76$

Hence we conclude that the variation due to rows and columns is not significant but the treatments, *i.e.*, different levels of clay, have significant effect on the yield. To determine which of the treatment pairs differ significantly, we have to calculate the critical difference (*C.D.*)

S.E. of difference between any two treatment means

$= s_E = \sqrt{(2/m)} = \sqrt{(2 \times 26{\cdot}0616/4)} = 3{\cdot}609$

$\therefore$ C.D. $= 3{\cdot}609 \times t_{0{\cdot}025}$ (for error *d.f.*)

$= 3{\cdot}609 \times 2{\cdot}447 = 8{\cdot}83.$

We now arrange the treatment means in their decreasing order of magnitude as given in the *Table 6·23*.

TABLE 6·23

Treatment	*Mean Yield*	$\lvert \Delta \equiv$ *Difference* $\rvert$
C	31·1250	1·625
D	29·5000	7·775
B	21·7260	14·025
A	7·7000	

We, therefore, conclude that :

(*i*) The difference between mean yields of *C* and *D* is not significant and they may therefore be regarded alike as regards their effect on yield. Similar argument holds for the pair *D* and *B*.

(*ii*) The treatments C and B are significantly different from each other as regards their effect on yields, since the difference between their mean yields, *viz.*, 9·4 exceeds the *C.D.* As such treatment C is to be preferred to treatment B. Similar argument holds for any other pair left.

(*b*) Efficiency of L.S.D. :

(*i*) Relative efficiency of L.S.D. over R.B.D. when *rows* are taken as blocks is :

$$\frac{s_C^2 + (m-1)\,s_E^2}{m\,s_E^2} = \frac{51{\cdot}7575 + 3 \times 26{\cdot}0616}{4 \times 26{\cdot}0616} = 1{\cdot}2465$$

Relative efficiency of L.S.D. over R.B.D. when columns are taken as blocks is

$$\frac{s_R^2 + (m-1)\,s_E^2}{m\,s_E^2} = \frac{86{\cdot}4375 + 3 \times 26{\cdot}0616}{4 \times 26{\cdot}0616} = 1{\cdot}5792$$

(*iii*) Relative efficiency of an L.S.D. over C.R.D. is :

$$\frac{s_R^2 + s_C^2 + (m-1)s_E^2}{(m+1)\,s_E^2} = \frac{116{\cdot}3798}{130{\cdot}3080} = 1{\cdot}6605$$

Hence, the gain by using Latin Square Design

(*i*) instead of R.B.D. is 25% when rows are taken as blocks and 58% when columns are taken as blocks.

(*ii*) instead of C.R.D. is 66%.

(*c*) *Missing observation.* Using (6·80), we get the estimate for missing value

$$\hat{x} = \frac{4(99{\cdot}8 + 70{\cdot}4 + 25{\cdot}4) - 2 \times 354{\cdot}8}{3 \times 2} = 12{\cdot}13$$

Statistical Analysis. As a result of replacing the missing figure 5·4 by its estimate 12·13, *Corrected or Adjusted S.S.* are obtained as follows :

$$\text{Raw S.S.} = 10{,}052{\cdot}08 - (5{\cdot}4)^2 + (12{\cdot}13)^2 = 10{,}170{\cdot}06$$

$$G^2 = (360{\cdot}2 - 5{\cdot}4 + 12{\cdot}13)^2 = (366{\cdot}93)^2 = 134637{\cdot}62$$

$$C.F. = G^2/16 = 8{,}414{\cdot}85$$

$$\therefore \quad \text{Total S.S.} = \text{R.S.S.} - \text{C.F.} = 10{,}170{\cdot}06 - 8{,}414{\cdot}85 = 1{,}755{\cdot}21$$

$$\sum R_i^2 = 33{,}473{\cdot}26 - (105{\cdot}2)^2 + (105{\cdot}2 - 5{\cdot}4 + 12{\cdot}13)^2 = 34{,}934{\cdot}54$$

$$\therefore \quad \text{S.S.R.} = \frac{34{,}934{\cdot}54}{4} - 8{,}414{\cdot}85 = 318{\cdot}79$$

$$\sum C_j^2 = 33{,}057{\cdot}10 - (75{\cdot}8)^2 + (75{\cdot}8 - 5{\cdot}4 + 12{\cdot}13)^2 = 34{,}122{\cdot}66$$

$$\therefore \quad \text{S.S.C.} = \frac{34122{\cdot}66}{4} - 8{,}414{\cdot}85 = 115{\cdot}81$$

$$\sum T_k^2 = 37{,}924{\cdot}50 - (30{\cdot}8)^2 + (30{\cdot}8 - 5{\cdot}4 + 12{\cdot}13)^2 = 38{,}384{\cdot}36$$

Adjusting factor for treatment S.S. is [c.f. (6·80*a*)] :

$$\frac{(3 \times 25{\cdot}4 + 99{\cdot}8 + 70{\cdot}4 - 354{\cdot}8)^2}{(3 \times 2)^2} = \frac{(-108{\cdot}4)^2}{6 \times 6} = 326{\cdot}40$$

$$\therefore \quad \text{Adjusted S.S.T.} = \frac{38{,}384{\cdot}36}{4} - 8{,}414{\cdot}85 - 326{\cdot}40 = 854{\cdot}84$$

$$\text{Adjusted SSE} = \text{TSS} - \text{SSR} - \text{SSC} - \text{SST (Adjusted)}$$
$$= 1{,}755{\cdot}21 - 318{\cdot}79 - 115{\cdot}81 - 854{\cdot}84 = 465{\cdot}77$$

TABLE 6·24 : CORRECTED ANOVA FOR LSD (MISSING OBSERVATION)

Source of Variation	*d.f.*	*S.S.*	*Mean S.S.*	*Variance Ratio*
(1)	(2)	(3)	(4) = (3) ÷ (2)	*F*
Rows	3	318·79	106·26	$F_R = \frac{106·26}{93·15} = 1·14$
Columns	3	115·81	38·60	$F_c < 1$
Treatments (Adjusted)	3	854·84	284·95	$F_T = \frac{284·95}{93·15} = 3·06$
Error (Adjusted)	14 – 9 = 5*	465·77	93·15	
Total	15 – 1 = 14*	1,755·21		

Note. 1 *d.f.* is reduced for the *d.f.* of total S.S. and consequently for Error S.S. because one missing observation has been estimated and its estimated value is used in computing the various S.S.

Tabulated $F_{3,5}$ (0·05) = 5·41

Since the calculated values of F_R, F_c and F_T are less than the tabulated value, more of them is significant. Hence, the treatments do not differ significantly.

Remark. If the treatments had differed significantly in this case also, then we would have used the result in (6·81) in computing the Critical Difference (C.D.) for the difference of treatment means, one of which involves the missing observation.

Duncan's new multiple range test

In statistics, **Duncan's new multiple range test** (**MRT**) is a multiple comparison procedure developed by David B. Duncan in 1955. Duncan's MRT belongs to the general class of multiple comparison procedures that use the studentized range statistic q_r to compare sets of means.

David B. Duncan developed this test as a modification of the Student–Newman–Keuls method that would have greater power. Duncan's MRT is especially protective against false negative (Type II) error at the expense of having a greater risk of making false positive (Type I) errors. Duncan's test is commonly used in agronomy and other agricultural research.

The result of the test is a set of subsets of means, where in each subset means have been found not to be significantly different from one another.

Contents

Duncan's new multiple range test

- 5Criticism
  - 5.1Different approaches to the problem

Definition:

Assumptions:

1.A sample of observed means,which have been drawn independently from n normal populations with "true" means,respectively.

2.A common standard error .

This standard error is unknown, but there is available the usual estimate,which is independent of the observed means and is based on a number of degrees of freedom, denoted by . (More precisely, , has the property that is distributed as with degrees of freedom, independently of sample means).

The exact definition of the test is:

The difference between any two means in a set of n means is significant provided the range of each and every subset which contains the given means is significant according to

an level range test where , and is the number of means in the subset concerned.

Exception: The sole exception to this rule is that no difference between two means can be declared significant if the two means concerned are both contained in a subset of the means which has a non-significant range.

Procedure:

The procedure consists of a series of pairwise comparisons between means. Each comparison is performed at a significance level , defined by the number of means separating the two means compared (for separating means). The test are performed sequentially, where the result of a test determines which test is performed next.

The tests are performed in the following order:

The largest minus the smallest, the largest minus the second smallest, up to the largest minus the second largest; then the

second largest minus the smallest, the second largest minus the second smallest, and so on, finishing with the second smallest minus the smallest.

With only one exception, given below, each difference is significant if it exceeds the corresponding shortest significant range; otherwise it is not significant. Where the shortest significant range is the significant studentized range, multiplied by the standard error. The shortest significant range will be designated as , where is the number means in the subset. The sole exception to this rule is that no difference between two means can be declared significant if the two means concerned are both contained in a subset of the means which has a non-significant range.

An algorithm for performing the test is as follows:

1.Rank the sample means, largest to smallest.

2. For each sample mean, largest to smallest, do the following:

2.1 for each sample mean, (denoted), for smallest up to .

2.1.1 compare to critical value ,

2.1.2 if does not exceed the critical value,

The subset is declared not siginificantlly different:

2.1.2.1 Go to next iteration of loop 2.

2.1.3 Otherwise, keep going with loop 2.1

Critical values:

Duncan's multiple range test makes use of the studentized range distribution in order to determine critical values for comparisons between means. Note that different comparisons between means may differ by their significance levels- since the significance level is subject to the size of the subset of means in question.

Let us denote as the quantile of the studentized range distribution, with p

observations, and degrees of freedom for the second sample (see studentized range for more information). Let us denote as the standardized critical value, given by the rule:

If p=2

Else

The shortest critical range, (the actual critical value of the test) is computed as : .

For ->∞, a tabulation exists for an exact value of Q (see link). A word of caution is needed here: notations for Q and R are not the same throughout literature, where Q is sometimes denoted as the shortest significant interval, and R as the significant quantile for studentized range distribution (Duncan's 1955 paper uses both notations in different parts).

Numeric example:

Let us look at the example of 5 treatment means:

Duncan's new multiple range test

Treatments	T1	T2	T3	T4	T5
Treatment Means	9.8	15.4	17.6	21.6	10.8
Rank	5	3	2	1	4

With a standard error of , and (degrees of freedom for estimating the standard error). Using a known tabulation for Q, one reaches the values of :

Now we may obtain the values of the shortest significant range, by the formula:

Reaching:

Then, the observed differences between means are tested, beginning with the largest versus smallest, which would be compared with the least significant range Next, the difference of the largest and the second smallest is computed and compared with the least significant difference .

Duncan's new multiple range test

If an observed difference is greater than the corresponding shortest significant range, then we conclude that the pair of means in question is significantly different. If an observed difference is smaller than the corresponding shortest significant range, all differences sharing the same upper mean are considered insignificant, in order to prevent contradictions (differences sharing the same upper mean are shorter by construction).

For our case, the comparison will yield:

We see that there are significant differences between all pairs of treatments except (T3,T2) and (T5,T1). A graph underlining those means that are not significantly different is shown below:

T1 T5 T2 T3 T4

Protection and significance levels based on degrees of freedom:

The new multiple range test proposed by Duncan makes use of special protection levels

based upon degrees of freedom. Let be the protection level for testing the significance of a difference between two means; that is, the probability that a significant difference between two means will not be found if the population means are equal. Duncan reasons that one has p-1 degrees of freedom for testing p ranked mean, and hence one may conduct p-1 independent tests, each with protection level . Hence, the joint protection level is:

where

that is, the probability that one finds no significant differences in making p-1 independent tests, each at protection level , is , under the hypothesis that all p population means are equal. In general: the difference between any two means in a set of n means is significant provided the range of each and every subset, which contains the given means, is significant according to an –level range test, where p is the number of means in the subset concerned.

Duncan's new multiple range test

For , the protection level can be tabulated for various value of r as follows:

	Protection level	**probability of falsely rejecting**
p=2	0.95	0.05
p=3	0.903	0.097
p=4	0.857	0.143
p=5	0.815	0.185
p=6	0.774	0.226
p=7	0.735	0.265

Note that although this procedure makes use of the Studentized range, his error rate is neither on an experiment-wise basis (as with Tukey's) nor

on a per- comparisons basis. Duncan's multiple range test does not control the familywise error rate. See Criticism Section for further details.

Duncan Bayesian multiple comparison procedure:

Duncan (1965) also gave the first Bayesian multiple comparison procedure, for the pairwise comparisons among the means in a one-way layout. This multiple comparison procedure is different for the one discussed above.

Duncan's Bayesian MCP discusses the differences between ordered group means, where the statistics in question are pairwise comparison (no equivalent is defined for the property of a subset having 'significantly different' property).

Duncan modeled the consequences of two or more means being equal using additive loss functions within and across the pairwise comparisons. If one assumes the same loss

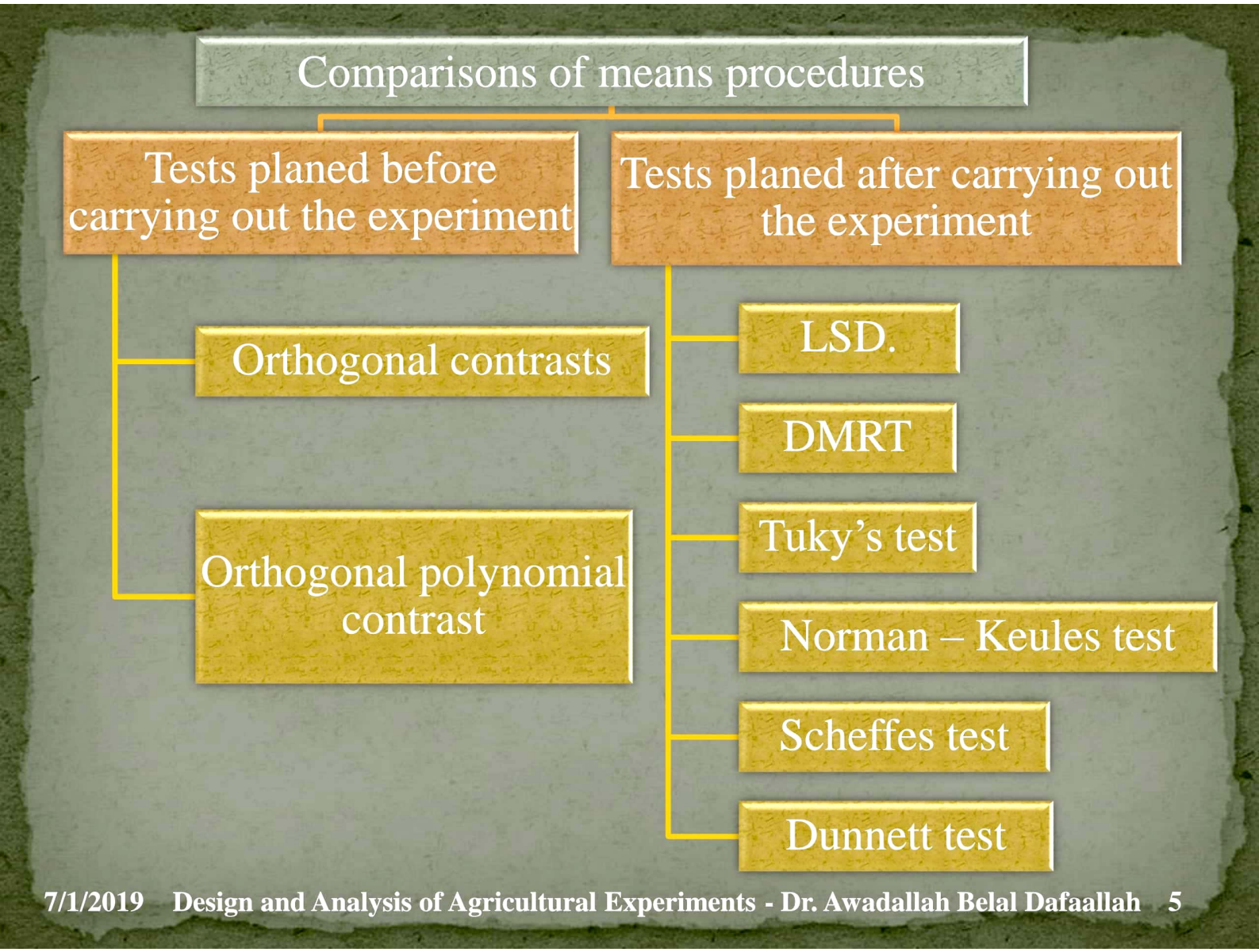
Comparisons of Means Procedures

Introduction:

- **Comparisons of means procedures** are also noun as means separation or multiple comparisons.
- They are not statistical designs.
- They are methods or means of comparing different statistical means or averages within the designs.

- In any design in AVOVA table, F-cal for treatments can be either significant or not significant.
- If F-cal for treatments is not significant (there are no real differences between the treatment means), therefore, there is no need to compare the treatment means.
- If F-cal for treatments is significant (there are real differences between the treatment means), therefore, there is a need to compare the treatment means.

- Then, F-test just shows without the details of whether there are real differences between the treatment means or not.
- To get the real differences between the treatment means we use means separation methods which could be classified into two types of tests.



Tests planed after carrying out the experiment:

- Least Significant Difference (LSD)
- Duncan's multiple range test (DMRT)

Least Significant Difference (LSD):

- LSD is considered by many researchers as the best method to compare multiple means for easy holding and then to the accuracy of the access to the correct results.
- This test also called protected least significant difference, because the Fisher (author) does not recommend using the test unless the F-cal for treatments is significant.
- LSD is original T-test which is used to compare differences between two means.

The use of LSD test:

- ✓ It is preferable to use LSD test when the f-cal for treatments is significant.
- ✓ It is not preferable to use LSD test to make all possible comparisons between treatment means.
- ✓ It is preferable to use LSD test to compare the mean of control treatment with the rest of the treatment means.

An example:

- With reference to the example, where the aim is to study the effect BAP on micro-propagation of Papaya (*Carica papaya* L.).
- After experiment carried out insuring that F-cal for treatments is significant, the researcher decided to use LSD.

Table. 1 Effect BAP on Micro-propagation of Papaya (*Carica papaya* L.)

Concentrations of BAP (mg/l)	0.0 mg/l	0. 5 mg/l	1.0 mg/l	1.5 mg/l	2.0 mg/l
Mean of root length (cm)	12.75	13.25	14.00	15.75	17.50
SE±			0.566		
CV %			7.73		

- Number of treatments = 5
- Number of replicates = 4
- Degree of freedom = 15
- Mean squares = 1.283

Steps for LSD test:

Lest significant difference is calculated from the following equation:

$$L.S.D = t_{(1-\alpha)(v)} \sqrt{\frac{2M.S.E.}{r}}$$

Where:

L. S. D	Lest significant difference
$t_{(1-\alpha)(v)}$	T value
$\sqrt{\frac{2M.S.E.}{r}}$	Standard error of differences between two means

1) Get T value from table of T-distribution with:

- Probability level = 1% or 5%
- Degree of freedom for error = 15

$$t_{(1-0.95)(v)} = 2.131$$

Table of T-distribution

Degrees of Freedom	Probability, p			
	0.1	0.05	0.01	0.001
1	6.31	12.71	63.66	636.62
2	2.92	4.30	9.93	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.89	2.37	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59
11	1.80	2.20	3.11	4.44
12	1.78	2.18	3.06	4.32
13	1.77	2.16	3.01	4.22
14	1.76	2.14	2.98	4.14
15	1.75	2.13	2.95	4.07

2) Calculate standard error of differences between two means.

$$\sqrt{\frac{2M.S.E.}{r}} = \sqrt{\frac{2 * 1.283}{4}} = 0.801$$

3) Calculate lest significant difference:

$$L.S.D = t_{(1-\propto)(v)} \sqrt{\frac{2M.S.E.}{r}}$$

$$= 2.131 * 0.801 = 1.71$$

4) Organize or arrange treatment means ascending or descending.

5) Table of mean differences.

6) Compare differences with LSD value.

Any mean difference greater or equal LSD value considered significant

Treatment number	Concentration of BAP	Means arranged descending	Mean differences			
			T_i-T_1	T_i-T_2	T_i-T_3	T_i-T_4
5	2.0 mg/l	17.50	4.75*	4.25*	3.50*	1.75*
4	1.5 mg/l	15.75	3.00*	2.50*	1.75*	
3	1.0 mg/l	14.00	1.25^{Ns}	0.75^{Ns}		
2	0.5 mg/l	13.25	0.50^{Ns}			
1	0.0 mg/l	12.75				

7) Summarize the mean differences using common line method or letter method.

Common line method:

Concentrations of BAP (mg/l)	2.0 mg/l	1.5 mg/l	1.0 mg/l	0.5 mg/l	0.0 mg/l
Mean of root length (cm)	17.50	15.75	14.00	13.25	12.75
SE±			0.566		
CV %			7.73		

* Differences which are not significant are given a common line.

Letter method:

Concentrations of BAP (mg/l)	2.0 mg/l	1.5 mg/l	1.0 mg/l	0.5 mg/l	0.0 mg/l
Mean of root length (cm)	17.50 a	15.75 b	14.00 c	13.25 c	12.75 c
SE±			0.566		
CV %			7.73		

* Differences which are not significant are given a different letters.

8) Results and discussion:

- BAB concentration of 2.0 mg/l, significantly, increased the root length of Papaya trees as compared with the rest of the concentrations.
- BAB concentration of 1.5 mg/l, significantly, increased the root length of Papaya trees as compared with the concentration 0.0, 0.5 and 1.0 mg/l.
- There are no significant differences in the root length of Papaya trees between BAB concentration of 0.0, 0.5 and 1.0 mg/l.

Appendix-III

parametric test for analysis of variance are also illustrated in the last chapter. We begin with the description and illustration of some important gap tests for use subsequent to the analysis of variance.

4.1 The Least Significant Difference (LSD) Test (extension of t-test)

F-test is used usually when there are more than two groups to be compared. This test tells us about the overall significant difference, if any, but does not tell us which one group is different from which other group. In such cases we have to resort to t-test or some other derived techniques. Least Significant Difference (LSD) test is one such technique. This is basically a Student's t-test using a pooled error variance (MS_W or within-group variance) computed in analysis of variance technique. The standard error of difference between the two means used in t-test i. e. (σ_D) is replaced here with error variance used in the F-test i. e. (S^2 or MS_W)

$$t = (M_1 - M_2)\ \sigma_D$$

$\therefore t \times \sigma_D = M_1 - M_2 =$ least significant difference and $\sigma D = \sqrt{2MS_W / n}$ where n = number of observations per mean to be compared (when n is same in all groups)

$\therefore LSD = t \times \sqrt{2MS_W / n}$

(When n_1 is different from n_2, $\sigma_D = \sqrt{MS_W / n_1 + MS_W / n_2}$

We can determine LSD at .05 or .01 level from the tabulated t value at .05 or .01 level for df = df for MS_W

Illustration

We have followed data of an agricultural experiment on some yield with six treatment means 28·8, 24.0, 14.6, 19.9, 13.3 and 18.7 made up by five observations in each treatment. The

F-test with error variance $MS_w = 11.79$ shows significant difference.

$$\text{LSD at } 5\% = t_{.05} \times \sqrt{2MS_w/n} = 2.064\sqrt{2.(11.79)/5} = 4.5$$

$$\text{LSD at } 1\% = t_{.01} \times \sqrt{2MS_w/n} = 2.797\sqrt{2(11.79)/5} = 6.1$$

(given $t_{.05} = 2.064$ and $t_{.01} = 2.797$ for df = df for $MS_w = 24$ in above example.

Thus, any two treatments with a difference equal to or more than above value are said to be statistically significantly different, Thus observed differences between three orthogonal comparisons, viz. $\bar{x}_1 - \bar{x}_2 = 4.8$, $\bar{x}_3 - \bar{x}_4 = 5.3$, and $\bar{x}_5 - \bar{x}_6 = 5.4$ are all significant at 5% though not at 1% level; other pairs do not differ significantly.

This LSD is useful for making orthogonal (independent) or even non-independent comparisons (in which same mean is compared more than once with other means). However, it is commonly misused when we make comparisons suggested by data, not initially planned.

For example, it can be shown that with three treatments the observed value of t for the greatest difference will exceed the tabulated 5% level about 13% of the time; with six treatments the figure is 40%, with ten treatments 60% and with twenty treatments 90% of the time. Thus when an experimenter is making a t-test at 5% level, he actually tests at 13% level for three treatments, 40% level for six treatments and so on. Hence it is not desirable to make more unplanned comparisons, suggested latter by the data. In such cases, especially to make multiple and non-independent comparisons, other tests such as Duncan's multiple-range test, Tukey's w procedure, etc. have been developed.

4.2 Duncan's New Multiple-Range Test

In 1951 Duncan developed a multiple comparisons test to compare each treatment mean with every other treatment

Tukey's range test

Tukey's range test, also known as the **Tukey's test**, **Tukey method**, **Tukey's honest significance test**, or **Tukey's HSD (honestly significant difference) test**,[1] is a single-step multiple comparison procedure and statistical test. It can be used to find means that are significantly different from each other.

Named after John Tukey,[2] it compares all possible pairs of means, and is based on a studentized range distribution (q) (this distribution is similar to the distribution of t from the t-test. See below).[3] The Tukey HSD tests should not be confused with the Tukey Mean Difference tests (also known as the Bland–Altman diagram).

Tukey's test compares the means of every treatment to the means of every other treatment; that is, it applies simultaneously to the set of all pairwise comparisons

and identifies any difference between two means that is greater than the expected standard error. The confidence coefficient for the set, when all sample sizes are equal, is exactly for any . For unequal sample sizes, the confidence coefficient is greater than 1 − α. In other words, the Tukey method is conservative when there are unequal sample sizes.

Contents

Assumptions:

1. The observations being tested are independent within and among the groups.
2. The groups associated with each mean in the test are normally distributed.
3. There is equal within-group variance across the groups associated with each mean in the test (homogeneity of variance).

The test statistic:

Tukey's test is based on a formula very similar to that of the *t*-test. In fact, Tukey's test is essentially a *t*-test, except that it corrects for family-wise error rate.

The formula for Tukey's test is:

where Y_A is the larger of the two means being compared, Y_B is the smaller of the two means being compared, and SE is the standard error of the sum of the means.

This q_s value can then be compared to a q value from the studentized range distribution. If the q_s value is *larger* than the critical value q_α obtained from the distribution, the two means are said to be significantly different at level .[3]

Since the null hypothesis for Tukey's test states that all means being compared are from the same population (i.e. $\mu_1 = \mu_2 = \mu_3 = \ldots = \mu_k$), the means should be normally distributed (according to the central limit theorem). This gives rise to the normality assumption of Tukey's test.

The studentized range (q) distribution[edit]

The Tukey method uses the studentized range distribution. Suppose that we take a sample of size n from each of k populations with the same normal distribution $N(\mu, \sigma^2)$ and suppose that $_{\min}$ is the smallest of these sample means and $_{\max}$ is the largest of these sample means, and suppose S^2 is the pooled sample variance from these

samples. Then the following random variable has a Studentized range distribution.

This value of q is the basis of the critical value of q, based on three factors:

1. α (the Type I error rate, or the probability of rejecting a true null hypothesis)
2. k (the number of populations)
3. df (the number of degrees of freedom ($N - k$) where N is the total number of observations)

The distribution of q has been tabulated and appears in many textbooks on statistics. In some tables the distribution of q has been tabulated without the factor. To understand which table it is, we can compute the result for $k = 2$ and compare it to the result of the Student's t-distribution with the same degrees of freedom and the same α. In

addition, R offers a cumulative distribution function (ptukey) and a quantile function (qtukey) for q.

Confidence limits:

The Tukey confidence limits for all pairwise comparisons with confidence coefficient of at least $1 - \alpha$ are

Notice that the point estimator and the estimated variance are the same as those for a single pairwise comparison. The only difference between the confidence limits for simultaneous comparisons and those for a single comparison is the multiple of the estimated standard deviation.

Also note that the sample sizes must be equal when using the studentized range approach. is the standard deviation of the entire design, not just that of the two groups being compared. It is possible to

work with unequal sample sizes. In this case, one has to calculate the estimated standard deviation for each pairwise comparison as formalized by Clyde Kramer in 1956, so the procedure for unequal sample sizes is sometimes referred to as the **Tukey–Kramer method** which is as follows:

where n_i and n_j are the sizes of groups i and j respectively. The degrees of freedom for the whole design is also applied.

Source :

1. S.C. Gupta and V.K. Kapoor : Fundamental of Applied Statistics – Sultan Chand & Sons, Fourth Edition, 2015.
2. Panneer Selvam: Design And Analysis of Experiments, Prentice Hall.