FUNDAMENTALS OF DESIGN OF EXPERIMENTS

1. Introduction

Any scientific investigation involves formulation of certain assertions (or hypotheses) whose validity is examined through the data generated from an experiment conducted for the purpose. Thus experimentation becomes an indispensable part of every scientific endeavour and designing an experiment is an integrated component of every research programme. Three basic techniques fundamental to designing an experiment are *replication*, *local control* (*blocking*), and *randomization.* Whereas the first two help to increase precision in the experiment, the last one is used to decrease bias. These techniques are discussed briefly below.

Replication is the repetition of the treatments under investigation to different experimental units. Replication is essential for obtaining a valid estimate of the experimental error and to some extent increasing the precision of estimating the pairwise differences among the treatment effects. It is different from *repeated measurements*. Suppose that the four animals are each assigned to a feed and a measurement is taken on each animal. The result is four independent observations on the feed. This is *replication*. On the other hand, if one animal is assigned to a feed and then measurements are taken four times on that animal, the measurements are not independent. We call them *repeated measurements*. The variation recorded in repeated measurements taken at the same time reflects the variation in the measurement process, while variation recorded in repeated measurements taken over a time interval reflects the variation in the single animal's responses to the feed over time. Neither reflects the variation in independent animal's responses to feed. We need to know about the latter variation in order to generalize any conclusion about the feed so that it is relevant to all similar animals.

For inferences to be broad in scope, it is essential that the experimental conditions should be rather varied and should be representative of those to which the conclusions of the experiment are to be applied. However, an unfortunate consequence of increasing the scope of the experiment is an increase in the variability of response. Local control is a technique that can often be used to help deal with this problem.

Blocking is the simplest technique to take care of the variability in response because of the variability in the experimental material. To block an experiment is to divide, or partition, the observations into groups called blocks in such a way that the observations in each block are collected under relatively similar experimental conditions. If blocking is done well, the comparisons of two or more treatments are made more precisely than similar comparisons from an unblocked design.

The purpose of randomization is to prevent systematic and personal biases from being introduced into the experiment by the experimenter. A random assignment of subjects or experimental material to treatments prior to the start of the experiment ensures that

observations that are favoured or adversely affected by unknown sources of variation are observations "selected in the luck of the draw" and not systematically selected.

Lack of a random assignment of experimental material or subjects leaves the experimental procedure open to experimenter bias. For example, a horticulturist may assign his or her favourite variety of experimental crop to the parts of the field that look the most fertile, or a medical practitioner may assign his or her preferred drug to the patients most likely to respond well. The preferred variety or drug may then appear to give better results no matter how good or bad it actually is.

Lack of random assignment can also leave the procedure open to systematic bias. Consider, for example, an experiment conducted to study the effect of drugs in controlling the blood pressure. There are three drugs available in the market that can be useful for controlling the diastolic blood pressure. There are 12 patients available for experimentation. Each drug is given to four patients. If the allotment of drugs to the patients is not random, then it is quite likely that the experimenter takes four observations on drug 1 from the four patients on whom the onset of the disease is recent; the four observations on drug 2 are taken on four patients on whom the disease is 5-6 years old; and the four observations on drug 3 are taken on four patients on whom the disease is chronic in nature. This arrangement of treatments on patients is also likely if the assignment of drugs to the patients is made randomly. However, deliberately choosing this arrangement could well be disastrous. Duration of illness could be a source of variation and, therefore, response to drug 1 would be better as compared to drug 2 and drug 3. This could naturally lead to the conclusion that drug 1 gives a better response to control blood pressure as compared to drug 2 and drug 3.

There are also analytical reasons to support the use of a random assignment. The process of *randomization ensures independence of observations*, which is necessary for drawing valid inferences by applying suitable statistical techniques. It helps in making objective comparison among the treatment effects. The interested reader is referred to Kempthorne (1977) and Dean and Voss (1999).

To understand the meaning of randomization, consider an experiment to compare the effects on blood pressure of three exercise programmes, where each programme is observed four times, giving a total of 12 observations. Now, given 12 subjects, imagine making a list of all possible assignments of the 12 subjects to the three exercise programs so that 4 subjects are assigned to each program. (There are 12! /(4!4!4!), or 34,650 ways to do this). If the assignment of subjects to programs is done in such a way that every possible assignment has the same chance of occurring, then the assignment is said to be a completely random assignment. Completely randomized designs discussed in section 3, are randomized in this way. It is indeed possible that a random assignment itself could lead to the order 1,1,1,1, 2,2,2,2, 3,3,3,3. If the experimenter expressly wishes to avoid certain assignments, then a different type of design should be used. An experimenter should not look at the resulting assignment, decide that it does not look very random, and change it.

The data generated through designed experiments exhibit a lot of variability. Even experimental units (plots) subjected to same treatment give rise to different observations

thus creating variability. The statistical methodologies, in particular the theory of linear estimation, enables us to partition this variability into two major components. The first major component comprises of that part of the total variability to which we can assign causes or reasons while the second component comprises of that part of the total variability to which we cannot assign any cause or reason. This variability arises because of some factors unidentified as a source of variation. Howsoever careful planning is made for the experimentation, this component is always present and is known as experimental error. The observations obtained from experimental units identically treated are useful for the estimation of this experimental error. Ideally one should select a design that will give experimental error as small as possible. There is, though, no rule of thumb to describe what amount of experimental error is small and what amount of it can be termed as large. A popular measure of the experimental error is the Coefficient of Variation (CV). The other major component of variability is the one for which the causes can be assigned or are known. There is always a deliberate attempt on the part of the experimenter to create variability by the application of several treatments. So treatment is one component in every designed experiment that causes variability. If the experimental material is homogeneous and does not exhibit any variability then the treatments are applied randomly to the experimental units. Such designs are known as zero-way elimination of heterogeneity designs or completely randomized designs (CRD). Besides the variability arising because of the application of treatments, the variability present in the experimental material (plots) is the other major known source of variability. Forming groups called blocks containing homogeneous experimental units can account for this variability if the variability in the experimental material is in one direction only. Contrary to the allotment of treatments randomly to all the experimental units in a CRD, the treatments are allotted randomly to the experimental units within each block. Such designs are termed as one-way elimination of heterogeneity setting designs or the block designs. The most common block design is the randomized complete block (RCB) design. In this design all the treatments are applied randomly to the plots within each block. However, for large number of treatments the blocks become large if one has to apply all the treatments in a block, as desired by the RCB design. It may then not be possible to maintain homogeneity among experimental units within blocks. As such the primary purpose of forming blocks to have homogeneous experimental units within a block is defeated. A direct consequence of laying out an experiment in RCB design with large number of treatments is that the coefficient of variation (CV) of the design becomes large. This amounts to saying that the error sum of squares is large as compared to the sum of squares attributable to the model and hence, small treatment differences may not be detected as significant. It also leads to poor precision of treatment comparisons or estimation of any normalized treatment contrast. High CV of the experiments is a very serious problem in agricultural experimentation. Many experiments conducted are rejected due to their high CV values. It causes a great loss of the scarce experimental resources. It is hypothesized that the basic problem with high CV and poor precision of estimation of treatment contrasts is that the block variations are not significant (block mean square is small as compared to error mean square) in large number of cases. In another research project entitled **A Diagnostic Study of Design and Analysis of Field Experiments**, carried out at Indian Agricultural Statistics Research Institute (IASRI), New Delhi, 5420 experiments were retrieved from Agricultural Field Experiments Information System conducted using a RCB design and were analyzed.

The replication effects were found to be not significantly different at 5% level of significance in more than 75% of the cases. A close scrutiny of the results of 1186 experiments conducted in RCB design by the PDCSR, Modipuram on Research Stations during 1990-2001, revealed that the replication effect was not significant in 740 (62.39%) of the experiments. In the varietal trials conducted under the aegis of All India Co-ordinated Research Project on Rapeseed and Mustard, we analyzed the data from different initial varietal trials (IVT) and advanced varietal trials conducted at different locations. Data from a total of 30 locations were analyzed as per procedure of RCB design (design adopted). It was found that the replication effects are not significantly different in 21 (70%) experiments. In any experimentation, non-significant differences between block effects or a high value of CV may arise due to any of the following causes:

1. Bad management of the controllable factors during experimentation (managerial aspects).
2. Faulty formation of blocks (designing).
3. Lack of identification of ancillary information that could have been used as covariate (Analysis of covariance).

The first point may be taken care of if the experimenter is very cautious and experienced. Analysis of covariance is an analytical procedure and is very effective in controlling the experimental error although it has nothing to do with the designing of the experiment. The most important point which has to be taken care of during the allocation of treatments to different experimental units is to adopt the proper blocking techniques. Therefore, there is a strong need to effectively control the variation through blocking. This necessitates the use of incomplete block designs. A block design is said to be an incomplete block design if the design has at least one block that does not contain all the treatments. Some common incomplete block designs are balanced incomplete block (BIB) design, partially balanced incomplete block (PBIB) design including Lattice designs – square and rectangular, cyclic designs, alpha designs, etc. One may, however, argue that in these designs the purpose of demonstration of a variety effect in the field cannot be done as all the treatments are not appearing in adjacent piece of land. To overcome this problem it is recommended that resolvable block designs with smaller block sizes may be used.

A resolvable block design is a design in which the blocks can be grouped in such a fashion that each of the treatments occurs in each of the groups exactly once; in other words, each group is a complete replication. One particular class of resolvable incomplete block designs that has been recommended for varietal trials is the class of Lattice designs (square lattice and rectangular lattice). The limitation of these designs is that the varieties is $v = s^2$ or $v = s(s-1)$. Further, the block size in case of square lattice designs is s and in case of rectangular lattice designs is s-1. If the number of genotypes to be assessed does not satisfy these conditions, then either some additional genotypes may be added or exiting genotypes may be deleted. This limitation on the number of genotypes and the block size has been overcome by the introduction of alpha designs in the literature. It is now possible to obtain an alpha design for any composite number of genotypes and for any block size. Only restriction is that the block size must be a factor of number of genotypes. In other words, it is possible to obtain an alpha design in $v = sk$ genotypes, where k denotes the block size and s is a positive integer. A critical look at the experimentation in the NARS

reveals that α-designs have not found much favour from the experimenters. It may possibly be due to the fact that the experimenters find it difficult to lay their hands on α-designs. The construction of these designs is not easy. An experimenter has to get associated with a statistician to get a randomized layout of this design. For the benefit of the experimenters, a comprehensive catalogue of α-designs for $6 \le v(= sk) \le 150$, $2 \le r \le 5$, $3 \le k \le 10$ and $2 \le s \le 15$ has been prepared along with lower bounds to A- and D- efficiencies and generating arrays. The layout of these designs along with block contents has also been prepared.

In some experimental situations, the user may be interested in getting designs outside the above parametric range. To circumvent such situations, a β- Version of user friendly software module for the generation of α-designs has been developed. This module generates the alpha array along with lower bounds to A and D-efficiency. The α-array and the design is generated once the user enter the number of treatments (v), number of replications (r) and the block size (k). The module generates the design for any v, k, r provided v is a multiple of k. It also gives the block contents of the design generated.

Further, the variability in the experimental material may be in two directions and forming rows and columns can control this variability and the treatments are assigned to the cells. Each cell is assigned one treatment. For the randomization purpose, first the rows are randomized and then the columns are randomized. There is no randomization possible within rows and/or within columns. Such designs are termed as two-way elimination of heterogeneity setting designs or the row-column designs. The most common row-column design is the Latin square design (LSD). The other row-column designs are the Youden square designs, Youden type designs, Generalized Youden designs, Pseudo Youden designs, etc.

In the experimental settings just described, the interest of the experimenter is to make all the possible pairwise comparisons among the treatments. There may, however, be situations where some treatments are on a different footing than the others. The set of treatments in the experiment can be divided into two disjoint groups. The first group comprises of two or more treatments called the test treatments while the second group comprises of a single or more than one treatment called the control treatments or the controls. The single control situation is very common with the experimenters. The test treatments are scarce and the experimenter cannot afford to replicate these treatments in the design. Thus, the tests are singly replicated in the design. Such a design in tests is a disconnected design and we cannot make all the possible pairwise comparisons among tests. Secondly, we cannot estimate the experimental error from such a design. To circumvent these problems, the control treatment(s) is (are) added in each block at least once. Such a design is called an augmented design. There may, however, be experimental situations when the tests can also be replicated. In such a situation the tests are laid out in a standard design like BIB design, PBIB design including Lattice design – square and rectangular, cyclic design, alpha designs, etc. and the control(s) is (are) added in each block once (or may be more than once). In this type of experimental setting the interest of the experimenter is not to make all the possible pairwise comparisons among the treatments, tests and controls together. The experimenter is interested in making pairwise

comparisons of the tests with the controls only. The pairwise comparisons among the tests or the controls are of no consequence to the experimenter. These experiments are very popular with the experimenters, particularly the plant breeders.

Another very common experimental setting is the following: An experiment is laid out at different locations/sites or is repeated over years. The repetition of the experiments over locations or years becomes a necessity for observing the consistency of the results and determining the range of geographical adaptability. In these experiments, besides analyzing the data for the individual locations/sites or years, the experimenter is also interested in the combined analysis of the data. For performing combined analysis of data, first the data for each experiment at a given location/site or year is analyzed separately. It is then followed by testing the homogeneity of error variances using Barttlett's χ^2 -test. The details of the Bartlett's χ^2 -test are given in Example 3. It is same procedure as given in the lecture notes on Diagnostics and Remedial Measures with only difference that the estimated error variances S_i^2 are to be replaced by mean square error and $r_i - 1$ is to be replaced by corresponding error degrees of freedom. If the errors are homogeneous, then the combined analysis of data is carried out by treating the environments (locations/sites and/or years) as additional factors. If, however, the error variances are heterogeneous, then the data needs a transformation. A simple transformation is that the observations are divided by the root mean square error. This transformation is similar to Aitken's transformation. The transformed data is then analyzed in the usual manner. In both these cases, first the interaction between the treatments and environments is tested against the error. If the interaction is significant *i.e.* the interaction is present, then the significance of treatments is tested against the interaction mean square. If the interaction is non-significant *i.e.* interaction is absent then the treatments are tested against the pooled mean squares of treatments × environment interaction and error. This is basically for the situations where the experiment is conducted using a RCB design. However, in general if the interaction is absent, then one may delete this term from the model and carry out the analysis using a model without interaction term.

The group of experiments may be viewed as a nested design with locations/years as the bigger blocks and the experiments nested within blocks. For doing the combined analysis, the replication wise data of the treatments at each environment provide useful information. The treatment × site (or year) interactions can also be computed. However, if at each site, only the average value of the observations pertaining to each treatment is given then it is not possible to study the treatment × site (or year) interaction. The different sites or the years are natural environments. The natural environments are generally considered as a random sample from the population. Therefore, the effect of environment (location or year) is considered as random. All other effects in the model that involve the environment either as nested or as crossed classification are considered as random. The assumption of these random effects helps in identifying the proper error terms for testing the significance of various effects.

Some other experimental situations that can be viewed as groups of experiments are those in which it is difficult to change the levels of one of the factors. For example, consider an

experimental situation, where the experimenter is interested in studying the long-term effect of irrigation and fertilizer treatments on a given crop sequence. There are 12 different fertilizer treatments and three-irrigation treatments viz. continuous submergence, 1 day drainage and 3 day drainage. It is very difficult to change the irrigation levels. Therefore, the three irrigation levels may be taken as 3 artificially created environments and the experiment may be conducted using a RCB design with 12 fertilizer treatments with suitable number of replications in each of the 3 environments. The data from each of the three experiments may be analyzed individually and the mean square errors so obtained may be used for testing the homogeneity of error variances and combined analysis of data be performed.

In case of artificially created environments, the environment effect also consists of the effect of soil conditions in field experiments. Therefore, it s suggested that the data on some auxiliary variables may also be collected. These auxiliary variables may be taken as covariate in the analysis.

Besides Aitken's transformation described above, other commonly used transformations are the arcsine transformation, square root transformation and the logarithmic transformation. These transformations are particular cases of a general family of transformations, Box-Cox transformation. The transformations other than Aitken's transformation are basically useful for the analysis of experimental data from individual experiments.

So far we have discussed about the experimental situations when the factors are cross-classified, *i.e.,* the levels of one factor are experimented at all the levels of the other factor. In practical situations it may be possible that one factor is nested within another factor. The variability in the experimental material enables us to form the blocks supposed to comprise of experimental units that are homogeneous. But the experimental units within block may also exhibit variability that can be further controlled by forming sub blocks within blocks. For example, in hilly areas the long strips may form the big blocks while small strips within the long strips may constitute the sub blocks. As another example, the trees are the big blocks and position of the branches on the trees may form the sub blocks. Such designs are called nested designs. The combined analysis of data can also be viewed as a nested design. The sites (or years) may constitute the big blocks and the experiments are nested within each block. The combined analysis of data can also be carried out as a nested design.

The experimental error can be controlled in two ways. As described above, one way of controlling the error is through the choice of an appropriate design by controlling the variability among the experimental units. The other way is through sound analytical techniques. There is some variability present in the data that has not been taken care of or could not be taken care of through the designing of an experiment. Such type of variability can be controlled at the time of analysis of data. If some auxiliary information is available on each experimental unit then this information can be used as a covariate in the analysis of covariance. The covariance analysis results into further reduction in the experimental error. But the auxiliary variable that is being used as a covariate should be such that it is not affected by the application of the treatments. Otherwise a part of the variability will be

eliminated while making adjustments for the covariate. There may be more than one covariate also.

The above discussion relates to the experimental situations in which the treatment structure comprises of many levels of a single factor. There are, however, experimental settings in which there are several factors studied together in an experiment. Each factor has several levels. The treatments comprise of all the possible combinations of several levels of all the factors. Such experiments where several factors with several levels are tried and the treatments are the treatment combinations of all the levels of all the factors are known as factorial experiments. Factorial experiments can be laid out in a CRD, RCB design, LSD or any other design. Factorial experiments, in fact, correspond to the treatment structure only. Consider a 3×2×2 experiment in which three levels of *Nitrogen* denoted as n_0, n_1, n_2, two levels of *Phosphorous* denoted as p_0, p_1 and two levels of *Potash* denoted as k_0, k_1 are tried. The *12* treatment combinations are $n_0p_0k_0, n_0p_0k_1, n_0p_1k_0, n_0p_1k_1, n_1p_0k_0, n_1p_0k_1, n_1p_1k_0, n_1p_1k_1, n_2p_0k_0, n_2p_0k_1, n_2p_1k_0, n_2p_1k_1$. This experiment can be laid out in any design. The advantage of factorial experiments is that several factors can be studied in one experiment and, therefore, there is a considerable saving of resources. The second advantage is that the precision of comparisons is improved because of the hidden replication of the levels of the factors. In the *12* treatment combinations, each treatment appears only once. But the levels of *N* appear *three* times each, the levels of *P* and *K* appear *six* times each, respectively. These are hidden replications that help in improved precision. The third advantage is that besides studying the main effects of factors we can also study the interactions among factors. The interaction helps us in studying the effect of levels of a factor at constant level of the other factors.

When the number of factors and/or levels of the factors increase, the number of treatment combinations increase very rapidly and it is not possible to accommodate all these treatment combinations in a single homogeneous block. For example, a 2^7 factorial would have 128 treatment combinations and blocks of 128 plots are quite big to ensure homogeneity within them. In such a situation it is desirable to form blocks of size smaller than the total number of treatment combinations (incomplete blocks) and, therefore, have more than one block per replication. The treatment combinations are then allotted randomly to the blocks within the replication and the total number of treatment combinations is grouped into as many groups as the number of blocks per replication.

There are many ways of grouping the treatments into as many groups as the number of blocks per replication. It is known that for obtaining the interaction contrast in a factorial experiment where each factor is at two levels, the treatment combinations are divided into two groups. Such two groups representing a suitable interaction can be taken to form the contrasts of two blocks each containing half the total number of treatments. In such cases the contrast of the interaction and the block contrast become identical. They are, therefore, mixed up and cannot be separated. In other words, the interaction gets confounded with the blocks. Evidently the interaction confounded has been lost but the other interactions and main effects can now be estimated with better precision because of reduced block size. This device of reducing the block size by taking one or more interactions contrasts

identical with block contrasts is known as **confounding**. Preferably only higher order interactions with three or more factors are confounded, because these interactions are less important to the experimenter. As an experimenter is generally interested in main effects and two factor interactions, these should not be confounded as far as possible. The designs for such confounded factorials are incomplete block designs. However, usual incomplete block designs for single factor experiments cannot be adopted, as the contrasts of interest in two kinds of experiments are different. The treatment groups are first allocated at random to the different blocks. The treatments allotted to a block are then distributed at random to its different units. When there are two or more replications in the design and if the same set of interactions is confounded in all the replications, then confounding is called **complete** and if different sets of interactions are confounded in different replications, confounding is called **partial**. In complete confounding all the information on confounded interactions is lost. However, in partial confounding, the information on confounded interactions can be recovered from those replications in which these are not confounded. In some experimental situations, some factors require large plot sizes and the effect of these factors is obvious, the experimenter is interested in the main effects of other factor and interaction with high precision. Split plot designs are used for such experimental situations. If the experimenter is interested only in interaction of the two factors and both factors require large plot sizes, the strip plot designs may be used.

In factorial experiments, sometimes, due to constraint on resources and/or time it is not possible to have more than one replication of the treatment combinations. In these situations, a single replicated factorial experiment with or without blocking is used and the higher order interactions are taken as error. To make the exposition clear, consider an experiment that was conducted to study the effect of irrigation (three levels), nitrogen (5 levels), depth (3-6 depths), classes of soil particle sizes (3-5) on organic carbon in rice–wheat cropping system. For each factorial combination, there is only one observation, and the experimenter was interested in studying the main effects and two factor interactions. Therefore, the data was analyzed as per procedure of singly replicated factorial experiment by considering the 3 –factor/4 factor interactions as the error term. In some of the experimental situations, the number of treatment combinations becomes so large that even a single replication becomes difficult. The fractional factorial plans are quite useful for these experimental situations.

The above discussion relates to the experiments in which the levels or level combinations of one or more factors are treatments and the data generated from these experiments are normally analyzed to compare the level effects of the factors and also their interactions. Though such investigations are useful to have objective assessment of the effects of the levels actually tried in the experiment, this seems to be inadequate, especially when the factors are quantitative in nature and cannot throw much light on the possible effect(s) of the intervening levels or their combinations. In such situations, it is more realistic and informative to carry out investigations with the twin purpose:

a) To determine and to quantify the relationship between the response and the settings of a set of experimental factors.
b) To find the settings of the experimental factor(s) that produces the best value or the best set of values of the response(s).

If all the factors are quantitative in nature, it is natural to think the response as a function of the factor levels and data from quantitative factorial experiments can be used to fit the response surface over the region of interest. The special class of designed experiments for fitting response surfaces is called response surface designs.

Through response surface designs one can obtain the optimum combination of levels of input factors. However, there do occur experimental situations where a fixed quantity of inputs, may be same dose of fertilizer, same quantity of irrigation water or same dose of insecticide or pesticide etc. are applied. The fixed quantity of input is a combination of two or more ingredients. For example, fixed quantity of water may be a combination of different qualities of water sources or fixed quantity of nitrogen may be obtained from different sources. In a pesticide trial, a fixed quantity of pesticide may be obtained from four different chemicals. In these experiments the response is a function of the proportion of the ingredient in the mixture rather than the actual amount of the mixture. The experiments with mixture methodology are quite useful for these experimental situations.

Besides controlling the variability in the experimental material by a process of forming blocks, rows and columns, etc. termed as *local control*, there are other techniques. The analysis of covariance technique is one very important way of reducing the experimental error.

2. Contrasts and Analysis of Variance

The main technique adopted for the analysis and interpretation of the data collected from an experiment is the analysis of variance technique that essentially consists of partitioning the total variation in an experiment into components ascribable to different sources of variation due to the controlled factors and error. Analysis of variance clearly indicates a difference among the treatment means. The objective of an experiment is often much more specific than merely determining whether or not all of the treatments give rise to similar responses. For examples, a chemical experiment might be run primarily to determine whether or not the yield of the chemical process increases as the amount of the catalyst is increased. A medical experimenter might be concerned with the efficacy of each of several new drugs as compared to a standard drug. A nutrition experiment may be run to compare high fiber diets with low fiber diets. A plant breeder may be interested in comparing exotic collections with indigenous cultivars. An agronomist may be interested in comparing the effects of biofertilisers and chemical fertilisers. An water technologist may be interested in studying the effect of nitrogen with Farm Yard Manure over the nitrogen levels without farm yard manure in presence of irrigation.

The following discussion attempts to relate the technique of analysis of variance to provide hypothesis tests and confidence intervals for the treatment comparisons among the treatment effects.

2.1 Contrasts

Let $y_1, y_2, \ldots, y_n$ denote n observations or any other quantities. The linear function $C = \sum_{i=1}^{n} l_i y_i$, where l_i's are given number such that $\sum_{i=1}^{n} l_i = 0$, is called a *contrast* of $y_i's$.

Let $y_1, y_2, \ldots, y_n$ be independent random variables with a common mean μ and variance σ^2. The expected value of the random variable C is zero and its variance is $\sigma^2 \sum_{i-1}^{n} l_i^2$. In what follows we shall not distinguish between a contrast and its corresponding random variable.

Sum of squares (s.s.) of contrasts. The sum of squares due to the contrast C is defined as $C^2 / \sigma^{-2} Var(C) = C^2 / \left(\sum_{i=1}^{n} l_i^2 \right)$. Here σ^2 is unknown and is replaced by its unbiased estimate, *i.e. mean square error*. It is known that this square has a $\sigma^2 \chi^2$ distribution with one degree of freedom when the $y_i's$ are normally distributed. Thus the sum of squares due to two or more contrasts has also a $\sigma^2 \chi^2$ distribution if the contrasts are independent.

Multiplication of any contrast by a constant does not change the contrast. The sum of squares due to a contrast as defined above is not evidently changed by such multiplication.

Orthogonal contrasts. Two contrasts, $C_1 = \sum_{i=1}^{n} l_i y_i$ and $C_2 = \sum_{i=1}^{n} l_i y_i$ are said to be orthogonal if and only if $\sum_{i=1}^{n} l_i m_i = 0$. This condition ensures that the covariance between C_1 and C_2 is zero.

When there are more than two contrasts, they are said to be mutually orthogonal if they are orthogonal pair wise. For example, with four observations y_1, y_2, y_3, y_4, we may write the following three mutually orthogonal contrasts:

(i) $y_1 + y_2 - y_3 - y_4$

(ii) $y_1 - y_2 - y_3 + y_4$

(iii) $y_1 - y_2 + y_3 - y_4$

The sum of squares due to a set of mutually orthogonal contrasts has a $\sigma^2 \chi^2$ distribution with as many degrees of freedom as the number of contrasts in the set.

Maximum number of orthogonal contrasts. Given a set of *n* values $y_1, y_2, \cdots, y_n$, the maximum number of mutually orthogonal contrasts among them is *n - 1*. One way of writing such contrasts is to progressively introduce the values as below:

(i) $y_1 - y_2$

(ii) $y_1 + y_2 - 2y_3$

: :

: :

(n) $y_1 + y_2 + \cdots + y_{n-1} - (n-1) y_n$.

Another set of orthogonal contrasts for values of *n* is available in the Tables for Biological, Agricultural and Medical Research prepared by Fisher and Yates (1963) under the name of orthogonal polynomials.

To be specific about treatment effects let $\sum l_i t_i$ denote a treatment contrast, $\sum_i l_i = 0$. The BLUE of $\sum l_i t_i$ is $\sum_i l_i \hat{t}_i$ and its variance is denoted by $Var(\sum_i l_i \hat{t}_i)$, where t_i is the parameter pertaining to the treatment effect *i*. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is $\left(\sum_i l_i \hat{t}_i\right)^2 / \sigma^{-2} \hat{Var}\left(\sum_i l_i \hat{t}_i\right)$ where σ^2 is the error variance estimated by the error mean squares, MSE. The significance of the contrast can be tested using the statistic

$$t = \frac{\sum_i l_i \hat{t}_i}{\sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)}}$$

which follows the Student's t-distribution with degrees of freedom same as that of error. The null hypothesis is rejected at $\alpha\%$ level of significance if the tabulated value of $t_{(1-\alpha/2, edf)}$ is greater than computed t-value. Here *edf* represents the error degrees of freedom. F-test can be used instead of t-test using the relationship that $t^2_{n_1} = F_{1,n_1}$. Contrasts of the type $t_i - t_m$ in which experimenters are often interested are obtainable from $\sum_i l_i t_i$ by putting $l_i = 1, l_m = -1$ and zero for the other *l's*. These contrasts are called as elementary contrasts and are useful for pairwise comparisons.

Besides hypothesis testing, the experimenter may also be interested in obtaining a confidence interval. In the sequel, we shall give a formula for a confidence interval for an individual contrast. If confidence intervals for more than one contrast are required, then the multiple comparison methods should be used instead. A-*100 (1 - α)%* confidence interval for the contrast $\sum l_i t_i$ is

$$\sum l_i \hat{t}_i - t_{edf,\alpha/2} \sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)} \le l_i t_i \le \sum l_i \hat{t}_i + t_{edf,\alpha/2} \sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)}.$$

We can write this more succinctly as

$$\sum l_i t_i \in \left(\sum l_i \hat{t}_i \pm t_{edf,\alpha/2} \sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)}\right)$$

where the symbol $\pm$ denotes that the upper limit of the interval is calculated using + and the lower limit using - and *edf* is the number of degrees of freedom for error. The symbol "$\sum l_i t_i \in$" mean that the interval includes the true value of contrast $\sum l_i t_i$ with *100(1 - α)%* confidence.

The outcome of a hypothesis test can be deduced from the corresponding confidence interval in the following way. The null hypothesis $H_0 : \sum_i l_i t_i = h$ will be rejected at significance level α in favor of the two-sided alternative hypothesis $H_1 : \sum_i l_i t_i \neq h$ if the corresponding confidence interval for $\sum_i l_i t_i$ fails to contain *h*.

So far we have discussed experimental situations where one is interested in a single treatment contrast. However, there may be situations when one is interested in a group of treatment contrasts $\mathbf{L}'\mathbf{t}$, where $\mathbf{L}'$ is a $p \times v$ matrix such that $\mathbf{L}'\mathbf{1} = \mathbf{0}$, Rank (**L**) = *p*, and **t** = $(t_1, t_2, \cdots, t_v)'$ is a $v \times 1$ vector of treatment effects. The sum of squares due to a set of treatment contrasts $\mathbf{L}'\mathbf{t}$ is $(\mathbf{L}'\hat{\mathbf{t}})'\left(\mathbf{L}'\mathbf{C}^{-}\mathbf{L}\right)^{-}\mathbf{L}'\hat{\mathbf{t}}$ and the dispersion matrix of $\mathbf{L}'\hat{\mathbf{t}}$, the best linear unbiased estimator of $\mathbf{L}'\mathbf{t}$, is D($\mathbf{L}'\hat{\mathbf{t}}$)= $\sigma^2\left(\mathbf{L}'\mathbf{C}^{-}\mathbf{L}\right)$ and **C** is the coefficient matrix of reduced normal equations for estimating the linear functions of treatment effects. The null hypothesis of interest say is $H_0 : \mathbf{L}'\mathbf{t} = \mathbf{0}$ against $H_1 : \mathbf{L}'\mathbf{t} \neq \mathbf{0}$. The null hypothesis H_0 is tested using the statistic F= $\dfrac{\text{SS(set of Contrasts)}}{\text{MSE}}$ with *p* and *edf* (error degrees of freedom) degrees of freedom. If $\mathbf{L}'$ comprises of a complete set of (*v-1*) linearly independent parametric functions, *i.e., p = v-1,* then we can get the treatment sum of squares as we get in the ANOVA table. For more details on contrast analysis, a reference may be made to Dean and Voss (1999).

In multi-factor experiments, the treatments are combinations of levels of several factors. In these experimental situations, the treatment sum of squares is partitioned into sum of squares due to main effects and interactions. These sums of squares can also be obtained through contrast analysis. The procedure of obtaining sum of squares due to main effects and interactions is discussed in the sequel.

2.2 Main Effects and Interactions

In general, let there be *n*-factors, say $F_1, F_2, ..., F_n$ and i^{th} factor has s_i levels, $i = 1, ..., n$. The $v = (\prod_{i=1}^{n} s_i)$ treatment combinations in the lexico-graphic order are given by $\mathbf{a}_1 \times \mathbf{a}_2 \times ... \times \mathbf{a}_n$ where $\times$ denotes the symbolic direct product and $\mathbf{a}_i' = (0,1,..., s_{i-1})$; $i = 1,2,..., n$. Renumber the treatment combinations from 1 to *v* and analyze the data as per procedure of general block designs for single factor experiments. The treatment sum of squares obtained from the ANOVA is now to be partitioned into

main effects and interactions. This can easily be done through contrast analysis. One has to define the set of contrasts for each of the main effects and interactions. Before describing the procedure of defining contrasts for main effects and interactions, we give some preliminaries. The total number of factorial effects (main effects and interactions) are $2^n - 1$. The set of main effects and interactions have a one-one correspondence with $\mathbf{\Omega}$, the set of all *n*-component non-null binary vectors. For example a typical *p*-factor interaction.

$F_{g_1}, F_{g_2}, ..., F_{g_p}$ $\left(1 \le g_1 \le g_2 \le ... \le g_p \le n, 1 \le p \le n\right)$ corresponds to the element $x = \left(x_1, ..., x_n\right)$ of $\mathbf{\Omega}$ such that $x_{g_1} = x_{g_2} = ... = x_{g_p} = 1$ and $x_u = 0$ for $u \ne g_1, g_2, ..., g_p$.

The treatment contrasts belonging to different interactions $F^x, x = \left(x_1, ..., x_n\right) \in \mathbf{\Omega}$ are given by

$\mathbf{P}^x \mathbf{t}$, where $\mathbf{P}^x = \mathbf{P}_1^{x_1} \otimes \mathbf{P}_2^{x_2} \otimes ... \otimes \mathbf{P}_n^{x_n}$

where $\mathbf{P}_i^{x_i} = \mathbf{P}_i$ if $x_i = 1$

$= \mathbf{1}'_{s_i}$ if $x_i = 0$

where $\mathbf{P}_i$ is a $\left(s_i - 1\right) \times s_i$ matrix of complete set of linearly independent contrasts of order s_i and $\mathbf{1}_{s_i}$ is a $s_i \times 1$ vector of ones. For example, if $s_i = 4$, then $\mathbf{P}_i = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 1 & 1 & -2 & 0 \\ 1 & 1 & 1 & -3 \end{bmatrix}$.

For sum of squares of these contrasts and testing of hypothesis, a reference may be made to section 2.1.

In the sequel we describe some basic designs.

3. Completely Randomized Design

Designs are usually characterized by the nature of grouping of experimental units and the procedure of random allocation of treatments to the experimental units. In a completely randomized design the units are taken in a single group. As far as possible the units forming the group are homogeneous. This is a design in which only randomization and replication are used. There is no use of local control here.

Let there be *v* treatments in an experiment and *n* homogeneous experimental units. Let the i^{th} treatment be replicated r_i times *(i = 1,2,…, v)* such that $\sum_{i=1}^{v} r_i = n$. The treatments are allotted at random to the units.

Normally the number of replications for different treatments should be equal as it ensures equal precision of estimates of the treatment effects. The actual number of replications is, however, determined by the availability of experimental resources and the requirement of

precision and sensitivity of comparisons. If the experimental material for some treatments is available in limited quantities, the numbers of their replication are reduced. If the estimates of certain treatment effects are required with more precision, the numbers of their replication are increased.

Randomization

There are several methods of random allocation of treatments to the experimental units. The *v* treatments are first numbered in any order from *1* to *v*. The *n* experimental units are also numbered suitably. One of the methods uses the random number tables. Any page of a random number table is taken. If *v* is a one-digit number, then the table is consulted digit by digit. If *v* is a two-digit number, then two-digit random numbers are consulted. All numbers greater than *v* including zero are ignored.

Let the first number chosen be n_1; then the treatment numbered n_1 is allotted to the first unit. If the second number is n_2 which may or may not be equal to n_1 then the treatment numbered n_2 is allotted to the second unit. This procedure is continued. When the i^{th} treatment number has occurred r_i times, $(i = 1,2,...,v)$ this treatment is ignored subsequently. This process terminates when all the units are exhausted.

One drawback of the above procedure is that sometimes a very large number of random numbers may have to be ignored because they are greater than *v*. It may even happen that the random number table is exhausted before the allocation is complete. To avoid this difficulty the following procedure is adopted. We have described the procedure by taking *v* to be a two-digit number.

Let *P* be the highest two-digit number divisible by *v*. Then all numbers greater than *P* and zero are ignored. If a selected random number is less than *v*, then it is used as such. If it is greater than or equal to *v*, then it is divided by *v* and the remainder is taken to the random number. When a number is completely divisible by *v*, then the random number is *v*. If *v* is an *n*-digit number, then *P* is taken to be the highest *n*-digit number divisible by *v*. The rest of the procedure is the same as above.

Alternative methods of random allocation

If random number tables are not available, treatments can be allotted by drawing *lots* as below. Let the number of the i^{th} treatment be written on r_i pieces of papers $(i = 1,2,...,v)$. The $\sum_{i=1}^{v} r_i = n$ pieces of papers are then folded individually so that the numbers written on them are not visible. These papers are then drawn one by one at random. The treatment that is drawn in the t^{th} draw is allotted to the t^{th} plot $(t = 1,2,...,n)$.

Random allocation is also possible by using a fair coin. Let there be five treatments each to be replicated four times. There are, therefore, *20* plots. Let these plots be numbered from *1* to *20* conveniently.

When a coin is tossed, there are two events that is, either the head comes up, or the tail. We denote the "head" by H and the "tail" by T. When the coin is tossed twice, there are four events, that is, both times head HH; first head next tail HT: first tail next head TH and both times tail TT. Similarly, when the coin is thrown three times, there are the following eight possible events:

HHH, HHT, HTH, HTT, THH, THT, TTH, TTT.

Similar events can be written easily for four or more number of throws of the coin.

The five treatments are now labeled not by serial numbers as earlier but by any five of the above eight events obtainable by tossing three coins. Let us use the first five events and omit THT, TTH and TTT.

A coin is now thrown three times and the event happened noted. If the event is any of the first five events described above, the treatment labeled by it is allotted to the first experimental unit. If the event happened is any of the last three, it is ignored. The coin is again tossed three times and this event is used to select a treatment for the second experimental unit. If the same event occurs more than once, we are not to reject it until the number of times it has occurred equals the number of replications of the treatment it represents. This process is continued till all the experimental units are exhausted.

Analysis

This design provides a one-way classified data according to levels of a single factor. For its analysis the following model is taken:

$$y_{ij} = \mu + t_i + e_{ij}\ , \qquad i = 1,\cdots,v;\ j = 1,\cdots r_i\ ,$$

where y_{ij} is the random variable corresponding to the observation y_{ij} obtained from the j^{th} replicate of the i^{th} treatment, μ is the general mean, t_i is the fixed effect of the i^{th} treatment and e_{ij} is the error component which is a random variable assumed to be normally and independently distributed with zero means and a constant variance σ^2.

Let $\sum_j y_{ij} = T_i \quad (i = 1,2,...,v)$ be the total of observations from i^{th} treatment. Let further $\sum_i T_i = G$. Correction factor ($C.F.$) $= G^2/n$.

Sum of squares due to treatments $= \sum_{i=1}^{v} \frac{T_i^2}{r_i} - C.F.$

Total sum of squares $= \sum_{i=1}^{v}\sum_{j=1}^{r_i} y_{ij}^2 - C.F.$

ANALYSIS OF VARIANCE

Sources of variation	Degrees of freedom (D.F.)	Sum of squares (S.S.)	Mean squares (M.S.)	F
Treatments	$v-1$	$SST = \sum_{i=1}^{v} \frac{T_i^2}{r_i} - C.F.$	$MST = SST / (v - 1)$	MST/MSE
Error	$n-v$	SSE = by subtraction	$MSE = SSE / (n - v)$	
Total	$n-1$	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis that the treatments have equal effects is tested by F-test where F is the ratio *MST / MSE* with *(v - 1)* and *(n - v)* degrees of freedom. We may then be interested to either compare the treatments in pairs or evaluate special contrasts depending upon the objectives of the experiment. This is done as follows:

For a completely randomized design, the BLUE of the treatment contrast $\sum l_i t_i$ is

$\sum_i l_i \hat{t}_i = \sum_i l_i \bar{y}_i$, where $\bar{y}_i = T_i / r_i$, $Var(\sum_i l_i \hat{t}_i) = \sigma^2 \sum_i \frac{l_i^2}{r_i}$, where σ^2 is the error variance estimated by the error mean squares, MSE. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is $\left(\sum_i l_i \bar{Y}_i \right)^2 / \sum_i \frac{l_i^2}{r_i}$

The significance of the contrast can be tested by *t* test, where

$$t = \frac{\sum_i l_i \bar{y}_i}{\sqrt{MSE \sum_i \frac{l_i^2}{r_i}}}$$

where $t_{1-\alpha/2,(n-v)}$ is the value of Student's *t* at the level of significance α and degree of freedom *(n - v)*. Contrasts of the type $t_i - t_m$ in which experimenters are often interested are obtainable from $\sum_i l_i t_i$ by putting $l_i = 1, l_m = -1$ and zero for the other *l's*. Such comparisons are known as *pairwise comparisons*.

Sometimes the levels of the treatment factors divide naturally into two or more groups, and the experimenter is interested in the difference of averages contrast that compares the average effect of one group with the average effect of the other group(s). For example, consider an experiment that is concerned with the effect of different colors of exam paper (the treatments) on students' exam performance (the response). Suppose that treatments *1*

and *2* represent the pale colors, white and yellow, whereas treatments *3*, *4* and *5* represent the darker colors, blue, green and pink. The experimenter may wish to compare the effects of light and dark colors on exam performance. One way of measuring this is to estimate the contrast $\frac{1}{2}(t_1 + t_2) - \frac{1}{3}(t_3 + t_4 + t_5),$, which is the difference of the average effects of the light and dark colors. The corresponding contrast coefficients are

$$\left[\frac{1}{2}, \frac{1}{2}, -\frac{1}{3}, -\frac{1}{3}, -\frac{1}{3}\right]$$

The BLUE of the above contrast would be $\frac{1}{2}\bar{y}_1 + \frac{1}{2}\bar{y}_2 - \frac{1}{3}\bar{y}_3 - \frac{1}{3}\bar{y}_4 - \frac{1}{3}\bar{y}_5$ with estimated standard error as $\sqrt{MSE(\frac{1}{4r_1} + \frac{1}{4r_2} + \frac{1}{9r_3} + \frac{1}{9r_4} + \frac{1}{9r_5})}$.

A $100(1-\alpha)\%$ confidence interval for the contrast $\sum_i l_i t_i$ is

$$\sum l_i \bar{y}_i - t_{n-v,\alpha/2}\sqrt{MSE\sum\frac{l_i^2}{r_i}} \le \sum l_i t_i \le \sum l_i \bar{y}_i + t_{n-v,\alpha/2}\sqrt{MSE\sum\frac{l_i^2}{r_i}}.$$

4. Randomized Complete Block Design

It has been seen that when the experimental units are homogeneous then a CRD should be adopted. In any experiment, however, besides treatments the experimental material is a major source of variability in the data. When experiments require a large number of experimental units, the experimental units may not be homogeneous, and in such situations CRD can not be recommended. When the experimental units are heterogeneous, a part of the variability can be accounted for by grouping the experimental units in such a way that experimental units within each group are as homogeneous as possible. The treatments are then allotted randomly to the experimental units within each group (or blocks). The principle of first forming homogeneous groups of the experimental units and then allotting at random each treatment once in each group is known as local control. This results in an increase in precision of estimates of the treatment contrasts, due to the fact that error variance that is a function of comparisons within blocks, is smaller because of homogeneous blocks. This type of allocation makes it possible to eliminate from error variance a portion of variation attributable to block differences. If, however, variation between the blocks is not significantly large, this type of grouping of the units does not lead to any advantage; rather some degrees of freedom of the error variance is lost without any consequent decrease in the error variance. In such situations it is not desirable to adopt randomized complete block designs in preference to completely randomized designs.

If the number of experimental units within each group is same as the number of treatments and if every treatment appears precisely once in each group then such an arrangement is called a ***randomized complete block design.***

Suppose the experimenter wants to study *v* treatments. Each of the treatments is replicated *r* times (the number of blocks) in the design. The total number of experimental units is, therefore, *vr*. These units are arranged into *r* groups of size *v* each. The error control measure in this design consists of making the units in each of these groups homogeneous.

The number of blocks in the design is the same as the number of replications. The *v* treatments are allotted at random to the *v* plots in each block. This type of homogeneous grouping of the experimental units and the random allocation of the treatments separately in each block are the two main characteristic features of randomized block designs. The availability of resources and considerations of cost and precision determine actual number of replications in the design.

Analysis

The data collected from experiments with randomized block designs form a two-way classification, that is, classified according to the levels of two factors, *viz.*, blocks and treatments. There are *vr* cells in the two-way table with one observation in each cell. The data are orthogonal and therefore the design is called an *orthogonal design.* We take the following model:

$$y_{ij} = \mu + t_i + b_j + e_{ij}\ , \qquad \begin{pmatrix} i = 1,2,...,v; \\ j = 1,2,...,r \end{pmatrix},$$

where y_{ij} denotes the observation from i^{th} treatment in j^{th} block. The fixed effects μ, t_i, b_j denote respectively the general mean, effect of the i^{th} treatment and effect of the j^{th} block. The random variable e_{ij} is the error component associated with y_{ij}. These are assumed to be normally and independently distributed with zero means and a constant variance σ^2.

Following the method of analysis of variance for finding sums of squares due to blocks, treatments and error for the two-way classification, the different sums of squares are obtained as follows: Let $\sum_j y_{ij} = T_i \ \ (i = 1,2,...,v)$ = total of observations from i^{th} treatment and $\sum_j y_{ij} = B_j \qquad j = 1,\cdots,r$ = total of observations from j^{th} block. These are the marginal totals of the two-way data table. Let further, $\sum_i T_i = \sum_j B_j = G.$

Correction factor (*C.F.*) = G^2/rv, Sum of squares due to treatments $= \sum_i \frac{T_i^2}{r} - C.F.,$

Sum of squares due to blocks $= \sum_j \frac{B_j^2}{v} - C.F.,$ Total sum of squares $= \sum_{ij} y_{ij}^2 - C.F.$

ANALYSIS OF VARIANCE

Sources of variation	Degrees of freedom (D.F.)	Sum of squares (S.S.)	Mean squares (M.S.)	F
Blocks	*r - 1*	$SSB = \sum_j \frac{B_j^2}{v} - C.F.$	*MSB = SSB / (r - 1)*	*MSB/MSE*
Treatments	*v - 1*	$SST = \sum_i \frac{T_i^2}{r} - C.F.$	*MST = SST / (v - 1)*	*MST/MSE*
Error	*(r - 1)(v - 1)*	*SSE = by subtraction*	*MSE = SSE / (v - 1)(r - 1)*	
Total	*vr - 1*	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis that the treatments have equal effects is tested by F-test, where F is the ratio *MST / MSE* with *(v - 1)* and *(v - 1)(r - 1)* degrees of freedom. We may then be interested to either compare the treatments in pairs or evaluate special contrasts depending upon the objectives of the experiment. This is done as follows:

Let $\sum l_i t_i$ denote a treatment contrast, $\sum_i l_i = 0$. The BLUE of $\sum l_i t_i$ is $\sum_i l_i \hat{t}_i = \sum_i l_i \bar{y}_i$, where $\bar{y}_i = T_i / r$, $Var(\sum_i l_i \hat{t}_i) = \frac{\sigma^2}{r} \sum_i l_i^2$, where σ^2 is estimated by the error mean squares, MSE. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is $\left(\sum_i l_i \bar{y}_i\right)^2 / \left(\sum_i l_i^2 / r\right)$. The significance of the contrast can be tested as per procedure described in sections 2 and 3. The *100 (1 - α)%* confidence interval for this contrast is

$$\sum l_i \bar{y}_i - t_{(v-1)(r-1),\alpha/2} \sqrt{MSE \sum l_i^2 / r} \le \sum l_i t_i \le \sum l_i \bar{y}_i + t_{(v-1)(r-1)-v,\alpha/2} \sqrt{MSE \sum l_i^2 / r}$$

As we know that the outcome of a hypothesis test can be deduced from the corresponding confidence interval in the following way. The null hypothesis $H_0 : \sum_i l_i t_i = 0$ will be rejected at significance level α in favor of the two-sided alternative hypothesis $H_1 : \sum_i l_i t_i \neq 0$ if the corresponding confidence interval for $\sum_i l_i t_i$ fails to contain *0*. The interval fails to contain *0* if the absolute value of $\sum l_i \bar{y}_i$ is bigger than

$t_{(v-1)(r-1),\alpha/2}\sqrt{MSE\sum_i l_i^2 / r}$. Therefore, all possible paired comparisons between treatment effects one may use the critical differences.

The critical difference for testing the significance of the difference of two treatment effects, say $t_i - t_j$ is $C.D. = t_{(v-1)(r-1),\alpha/2}\sqrt{2MSE/r}$, where $t_{(v-1)(r-1),\alpha/2}$ is the value of Student's *t* at the level of significance α and degree of freedom *(v - 1)(r - 1).* If the difference of any two-treatment means is greater than the C.D. value, the corresponding treatment effects are significantly different.

Example 4.1: An experiment was conducted to evaluate the efficacy of Londax 60 DF in transplanted rice as pre-emergent application as stand alone and as tank mix with grass partner against different weed flora. The weed counts were recorded. The details of the experiment are given below:

The weed Count in Rice

Treatment	Dose (gai/ha)	Replications		
		1	2	3
Londax 60 DF	30	72	60	59
Londax 60 DF	45	81	56	71
Londax 60 DF	60	66	49	56
Londax+ Butachlor	30+938	8	9	4
Londax + Butachlor	45+938	10	17	6
Londax+ Butachlor	60+938	4	8	3
Butachlor 50 EC	938	22	10	11
Pretilachlor 50 EC	625	4	8	10
Pyrazo.Eth.10 WP	100 g/acre	20	46	33
Untreated Control	-	79	68	84

Analyze the data and draw your conclusions.

Procedure and Calculations

We compute the following totals:

Treatments totals ($y_{i.}$)	Treatment means ($\bar{y}_{i.} = y_{i.}/b$)
$y_{1.}$ = 72 + 60 + 59 = 191	$\bar{y}_{1.}$ = 191/3 = 63.6667
$y_{2.}$ = 81 + 56 + 71 = 208	$\bar{y}_{2.}$ = 208/3 = 69.3333
$y_{3.}$ = 66 + 49 + 56 = 171	$\bar{y}_{3.}$ = 171/3 = 57.0000
$y_{4.}$ = 8 + 9+ 4 = 21	$\bar{y}_{4.}$ = 21/3 = 7.0000
$y_{5.}$ = 10 + 17 + 6 = 33	$\bar{y}_{5.}$ = 33/3 = 11.0000
$y_{6.}$ = 4 + 8 + 3 = 15	$\bar{y}_{6.}$ = 15/3 = 5.0000
$y_{7.}$ = 22 + 10 + 11 = 43	$\bar{y}_{7.}$ = 43/3 = 14.3333
$y_{8.}$ = 4 + 8 + 10 = 22	$\bar{y}_{8.}$ = 22/3 = 7.3333
$y_{9.}$ = 20 + 46 + 33 = 99	$\bar{y}_{9.}$ = 99/3 = 33.0000
$y_{10.}$ = 79 + 68 + 84 = 231	$\bar{y}_{10.}$ = 231/3 = 77.0000

Replication (or Blocks) Totals ($y_{.j}$)	Replication Means ($\bar{y}_{.j} = y_{.j} / v$)
$y_{.1} = 72 + \ldots + 79 = 366$	$\bar{y}_{.1} = 366/10 = 36.6$
$y_{.2} = 60 + \ldots + 68 = 331$	$\bar{y}_{.2} = 331/10 = 33.1$
$y_{.3} = 59 + \ldots + 84 = 337$	$\bar{y}_{.3} = 337/10 = 33.7$

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 1034.0000$.

Correction Factor = $(y_{..})^2 / vb = (1034)^2/30 = 35638.5333$

Sum of Squares due to Trees = $\sum_i y_{i.}^2 / b - C.F.$

$$= \left(191^2 + \cdots + 231^2\right)/3 - 35638.5333 = 23106.8$$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$$= \left(366^2 + 331^2 + 337^2\right)/10 - 35638.5333 = 70.0667.$$

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$$= 72^2 + 81^2 + \cdots + 84^2 - C.F. = 24343.4667.$$

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to Trees - Sum of Squares due to Replications = *24343.4667 – 70.0667 – 23106.8000* = 1166.6000.

We now form the following Analysis of Variance Table:

ANOVA

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Trees	9	23106.8000	2567.422	39.61	*0.000*
Due to Replications	2	70.0667	35.03335	0.54	*0.592*
Error	18	1166.6000	64.81111		
Total	29	24343.4667			

Critical Difference between any two tree means = $t_{\alpha, error\ d.f.} x\sqrt{2MSE / b}$

$$= 2.101 \times \sqrt{(2 \times 64.81111)/3} = 13.810$$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two trees effects:

				Mean	Treatment No.
			A	77.0000	10
		B	*A*	69.3330	2
		B	*A*	63.6670	1
		B		57.0000	3
	C			33.0000	9
D				14.3330	7
D				11.0000	5
D				7.3330	8
D				7.0000	4
D				5.0000	6

Suppose now that treatment numbers 1, 2, 3 and treatment numbers 4, 5, 6 form two groups as the treatments in group 1 are with Londax only where as group2 comprises of treatments in which Butachlor is added along with Londax. Our interest is in comparing the two groups. We shall have the following contrast to be estimated and tested:

1. $t_1 + t_2 + t_3 - t_4 - t_5 - t_6$.

Similarly, suppose the other contrasts to be estimated and tested are:

2. $t_1 + t_2 + t_3 + t_4 + t_5 + t_6 + t_7 + t_8 + t_9 - 9t_{10}$
3. $t_4 + t_5 + t_6 - 3t_7$

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
1	*1*	13944.5000	13944.5000	215.16	0.0001
2	*1*	6030.2815	6030.2815	93.04	0.0001
3	*1*	100.0000	100.0000	1.54	0.2301

Suppose now that the interest of the experimenter is to test certain hypothesis concerning the three treatments in the Group 1. The sum of squares for testing the equality of tree effects can be obtained by defining four mutually orthogonal contrasts as $t_1 - t_2$; $t_1 + t_2 - 2t_3$.

Using these sets of contrasts we get the following:

Sl. No.	D.F.	S.S.	M.S.	F	Pr > F
1	2	228.6667	114.3333	1.76	0.1997

Example 4.2: An initial varietal trial (Late Sown, irrigated) was conducted to study the performance of 20 new strains of mustard vis-a-vis four checks (Swarna Jyoti: ZC; Vardan: NC; Varuna: NC; and Kranti: NC) using a Randomized complete Block Design (RCB) design at Bhatinda with 3 replications. The seed yield in kg/ha was recorded. The details of the experiment are given below:

Yield in kg/ha

Strain	Code	Replications		
		1	2	3
RK-04-3	MCN-04-110	1539.69	1412.35	1319.73
RK-04-4	MCN-04-111	1261.85	1065.05	1111.36
RGN-124	MCN-04-112	1389.19	1516.54	1203.97
HYT-27	MCN-04-113	1192.39	1215.55	1157.66
PBR-275	MCN-04-114	1250.27	1203.97	1366.04
HUJM-03-03	MCN-04-115	1296.58	1273.43	1308.16
RGN-123	MCN-04-116	1227.12	1018.74	937.71
BIO-13-01	MCN-04-117	1273.43	1157.66	1088.20
RH-0115	MCN-04-118	1180.82	1203.97	1041.90
RH-0213	MCN-04-119	1296.58	1458.65	1250.27
NRCDR-05	MCN-04-120	1122.93	1065.05	1018.74
NRC-323-1	MCN-04-121	1250.27	926.13	1030.32
RRN-596	MCN-04-122	1180.82	1053.47	717.75
RRN-597	MCN-04-123	1146.09	1180.82	856.67
CS-234-2	**MCN-04-124**	1574.42	1412.35	1597.57
RM-109	MCN-04-125	914.55	972.44	659.87
BAUSM-2000	MCN-04-126	891.40	937.71	798.79
NPJ-99	MCN-04-127	1227.12	1203.97	1389.19
SWAN JYOTI (ZC)	**MCN-04-128**	1389.19	1180.82	1273.43
VARDAN (NC)	**MCN-04-129**	1331.31	1157.66	1180.82
PR-2003-27	MCN-04-130	1250.27	1250.27	1296.58
VARUNA (NC)	**MCN-04-131**	717.75	740.90	578.83
PR-2003-30	MCN-04-132	1169.24	1157.66	1111.36
KRANTI-(NC)	**MCN-04-133**	1203.97	1296.58	1250.27

Analyze the data and draw your conclusions.

Procedure and Calculations: We compute the following totals:

Treatment Total ($y_{i.}$)	**Treatment Mean ($\bar{y}_{i.} = y_{i.}/3$)**
$y_{1.} = 4271.77$	$\bar{y}_{1.} = 1423.92$
$y_{2.} = 3438.26$	$\bar{y}_{2.} = 1146.09$
$y_{3.} = 4109.70$	$\bar{y}_{3.} = 1369.90$
$y_{4.} = 3565.60$	$\bar{y}_{4.} = 1188.53$
$y_{5.} = 3820.28$	$\bar{y}_{5.} = 1273.43$
$y_{6.} = 3878.17$	$\bar{y}_{6.} = 1292.72$
$y_{7.} = 3183.57$	$\bar{y}_{7.} = 1061.19$
$y_{8.} = 3519.29$	$\bar{y}_{8.} = 1173.10$
$y_{9.} = 3426.68$	$\bar{y}_{9.} = 1142.23$
$y_{10.} = 4005.51$	$\bar{y}_{10.} = 1335.17$
$y_{11.} = 3206.72$	$\bar{y}_{11.} = 1068.91$
$y_{12.} = 3206.72$	$\bar{y}_{12.} = 1068.91$

Treatment Total ($y_{i.}$)	**Treatment Mean ($\bar{y}_{i.} = y_{i.}/3$)**
$y_{13.} = 2952.04$	$\bar{y}_{13.} = 984.01$
$y_{14.} = 3183.57$	$\bar{y}_{14.} = 1061.19$
$y_{15.} = 4584.34$	$\bar{y}_{15.} = 1528.11$
$y_{16.} = 2546.86$	$\bar{y}_{16.} = 848.95$
$y_{17.} = 2627.89$	$\bar{y}_{17.} = 875.96$
$y_{18.} = 3820.28$	$\bar{y}_{18.} = 1273.43$
$y_{19.} = 3843.44$	$\bar{y}_{19.} = 1281.15$
$y_{20.} = 3669.79$	$\bar{y}_{20.} = 1223.26$
$y_{21.} = 3797.13$	$\bar{y}_{21.} = 1265.71$
$y_{22.} = 2037.49$	$\bar{y}_{22.} = 679.16$
$y_{23.} = 3438.26$	$\bar{y}_{23.} = 1146.09$
$y_{24.} = 3750.82$	$\bar{y}_{24.} = 1250.27$

Replication (or Blocks) Totals ($y_{.j}$)	Replication Means ($\bar{y}_{.j} = y_{.j} / v$)
$y_{.1} = 29277.27$	$\bar{y}_{.1} =$ 29277.27/24=1219.89
$y_{.2} =$ 28061.73	$\bar{y}_{.2} =$ 28061.73/24=1169.24
$y_{.3} =$ 26545.19	$\bar{y}_{.3} =$ 26545.19/24=1106.05

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 83884.19$.

Correction Factor = $(y_{..})^2 / vb$ = $(83884.19)^2/72$ = 97730396.53

Sum of Squares due to treatments = $\sum_i y_{i.}^2 / b - C.F.$

$$= \left(4271.77^2 + \cdots + 3750.82^2\right)/3 - 97730396.53 = 2514143.05$$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$$= \left(29277.27^2 + 28061.73^2 + 26545.19^2\right)/24 - 97730396.53$$
$$= 156139.3283$$

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$$= 1539.69^2 + \cdots + 1250.27^2 - C.F. = 3133406.13.$$

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to treatments - Sum of Squares due to Replications = 3133406.13-2514143.05-156139.33=463123.75.

We now form the following Analysis of Variance Table:

ANOVA (Yield: Bhatinda)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	2514143.05	109310.57	10.86	*<0.0001*
Due to Replications	2	156139.33	78069.66	7.75	*0.0013*
Error	46	463123.75	10067.91		
Total	71	3133406.13			

R-Square	CV	Root MSE	Mean Yield
0.852198	8.612337	100.3390	1165.06

2. Critical Difference between any two tree means = $t_{\alpha, error\ d.f.} x\sqrt{2MSE / b}$

$$= 2.10290 \times \sqrt{(2 \times 10067.91)/3} = 164.91$$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two treatment effects:

Mean	Treatment No.		Mean	Treatment No.	
1528.11	15	A	1173.10	8	D E F
1423.93	1	A B	1146.10	23	E F G
1369.90	3	A B C	1146.10	2	E F G
1335.18	10	B C D	1142.22	9	E F G
1292.73	6	B C D E	1068.90	12	F G
1281.14	19	B C D E	1068.90	11	F G
1273.43	18	B C D E	1061.19	7	F G
1273.43	5	B C D E	1061.19	14	F G
1265.72	21	B C D E	984.02	13	G H
1250.27	24	C D E	875.97	17	H
1223.27	20	C D E F	848.96	16	H
1188.55	4	D E F	679.15	22	I

Suppose now that treatment numbers 19, 20, 22 and 24 are the checks and rest of the treatments are test entries. It is clear from the above Table that treatment 15 is significantly different from highest performing check. The above Table gives Our interest is in comparing the checks with new entries. We shall have the following contrast to be estimated and tested:

1. $4t_1 + 4t_2 + \cdots + 4t_{18} + 4t_{21} + 4t_{23} - 20t_{19} - 20t_{20} - 20t_{22} - 20t_{24}$.

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
Checks vs Entries	*1*	46128.89	46128.89	4.58	0.0376

Suppose the experimenter can test any other hypothesis of interest.

5. Latin Square Design

Latin square designs are normally used in experiments where it is required to remove the heterogeneity of experimental material in two directions. These designs require that the number of replications equal the number of ***treatments*** or ***varieties***.

Definition 1. A Latin square arrangement is an arrangement of v symbols in v^2 cells arranged in v rows and v columns, such that every symbol occurs precisely once in each row and precisely once in each column. The term v is known as the **order** of the Latin square.

If the symbols are taken as *A, B, C, D*, a Latin square arrangement of order 4 is as follows:

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

A Latin square is said to be in the ***standard form*** if the symbols in the first row and first column are in natural order, and it is said to be in the ***semi-standard form*** if the symbols of the first row are in natural order. Some authors denote both of these concepts by the term ***standard form***. However, there is a need to distinguish between these two concepts. The

standard form is used for randomizing the Latin-square designs, and the semistandard form is needed for studying the properties of the orthogonal Latin squares.

Definition 2. If in two Latin squares of the same order, when superimposed on one another, every ordered pair of symbols occurs exactly once, the two Latin squares are said to be **orthogonal**. If the symbols of one Latin square are denoted by Latin letters and the symbols of the other are denoted by Greek letters, the pair of orthogonal Latin squares is also called a **graeco-latin square**.

Definition 3. If in a set of Latin squares every pair is orthogonal, the set is called a set of **mutually orthogonal latin squares (MOLS)**. It is also called a **hypergraeco latin square.**

The following is an example of graeco latin square:

A	B	C	D		α	γ	δ	β		$A\alpha$	$B\gamma$	$C\delta$	$D\beta$
B	A	D	C		β	δ	γ	α		$B\beta$	$A\delta$	$D\gamma$	$C\alpha$
C	D	A	B		γ	α	β	δ		$C\gamma$	$D\alpha$	$A\beta$	$B\delta$
D	C	B	A		δ	β	α	γ		$D\delta$	$C\beta$	$B\alpha$	$A\gamma$

We can verify that in the above arrangement every pair of ordered Latin and Greek symbols occurs exactly once, and hence the two latin squares under consideration constitute a graecolatin square.

It is well known that the maximum number of MOLS possible of order v is $v - 1$. A set of $v - 1$ MOLS is known as a complete set of MOLS. Complete sets of MOLS of order v exist when v is a ***prime or prime power.***

Randomization

According to the definition of a Latin square design, treatments can be allocated to the v^2 experimental units (may be animal or plots) in a number of ways. There are, therefore, a number of Latin squares of a given order. The purpose of randomization is to select one of these squares at random. The following is one of the methods of random selection of Latin squares.

Let a $v \times v$ Latin square arrangement be first written by denoting treatments by Latin letters *A, B, C, etc.* or by numbers *1, 2, 3, etc.* Such arrangements are readily available in the ***Tables for Statisticians and Biometricians*** (Fisher and Yates, 1974). One of these squares of any order can be written systematically as shown below for a 5×5 Latin square:

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

For the purpose of randomization rows and columns of the Latin square are rearranged randomly. There is no randomization possible within the rows and/or columns. For example, the following is a row randomized square of the above 5×5 Latin square;

A	*B*	*C*	*D*	*E*
B	*C*	*D*	*E*	*A*
E	*A*	*B*	*C*	*D*
D	*E*	*A*	*B*	*C*
C	*D*	*E*	*A*	*B*

Next, the columns of the above row randomized square have been rearranged randomly to give the following random square:

E	*B*	*C*	*A*	*D*
A	*C*	*D*	*B*	*E*
D	*A*	*B*	*E*	*C*
C	*E*	*A*	*D*	*B*
B	*D*	*E*	*C*	*A*

As a result of row and column randomization, but not the randomization of the individual units, the whole arrangement remains a Latin square.

Analysis of Latin Square Designs

In Latin square designs there are three factors. These are the factors *P, Q,* and treatments. The data collected from this design are, therefore, analyzed as a three-way classified data. Actually, there should have been v^3 observations as there are three factors each at *v* levels. But because of the particular allocation of treatments to the cells, there is only one observation per cell instead of *v* in the usual three way classified orthogonal data. As a result we can obtain only the sums of squares due to each of the three factors and error sum of squares. None of the interaction sums of squares of the factors can be obtained. Accordingly, we take the model

$$Y_{ijs} = \mu + r_i + c_j + t_s + e_{ijs}$$

where y_{ijs} denotes the observation in the i^{th} row, j^{th} column and under the s^{th} treatment; $\mu, r_i, c_j, t_s \left(i, j, s = 1,2,...,v\right)$ are fixed effects denoting in order the general mean, the row, the column and the treatment effects. The e_{ijs} is the error component, assumed to be independently and normally distributed with zero mean and a constant variance, σ^2.

The analysis is conducted by following a similar procedure as described for the analysis of two-way classified data. The different sums of squares are obtained as below: Let the data be arranged first in a *row* $\times$ *column* table such that y_{ij} denotes the observation of (*i, j)*th cell of table.

Let $R_i = \sum_j y_{ij} = i^{th}\ row\ total\ (i = 1,2,...,v)$, $C_j = \sum_i y_{ij} = j^{th}\ column\ total\ (j = 1,2,...,v)$, T_s = sum of those observations which come from s^{th} treatment $(s = 1,2,\ldots,v)$, $G = \sum_i R_i = grand\ total$. Correction factor, $C.F. = \frac{G^2}{v^2}$. Treatment sum of squares = $\sum_s \frac{T_s^2}{v} - C.F.$, Row sum of squares = $\sum_i \frac{R_i^2}{v} - C.F.$, Column sum of squares = $\sum_j \frac{C_j^2}{v} - C.F.$

Analysis of Variance of $v \times v$ Latin Square Design

Sources of Variation	D.F.	S.S.	M.S.	F
Rows	$v-1$	$\sum_i \frac{R_i^2}{v} - C.F.$		
Columns	$v-1$	$\sum_j \frac{C_j^2}{v} - C.F.$		
Treatments	$v-1$	$\sum_s \frac{T_s^2}{v} - C.F.$	s_t^2	s_t^2 / s_e^2
Error	$(v-1)(v-2)$	By subtraction	s_e^2	
Total	v^2-1	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis of equal treatment effects is tested by *F*-test, where *F* is the ratio of treatment mean squares to error mean squares. If *F* is not significant, treatment effects do not differ significantly among themselves. If *F* is significant, further studies to test the significance of any treatment contrast can be made in exactly the same way as discussed for randomized block designs.

6. Illustrations for Combined Analysis of Data

Example 6.1: An initial varietal trial (Late Sown, irrigated) was conducted to study the performance of 20 new strains of mustard vis-a-vis four checks (Swarna Jyoti: ZC; Vardan: NC; Varuna: NC; and Kranti: NC) using a Randomized complete Block Design (RCB) design at four locations (Sriganganagar, Navgaon, Bhatinda and Hissar) with 2 replications at Sriganganagar and with 3 replications each at other 3 locations. The seed yield in kg/ha was recorded. The data pertaining to Bhatinda is given in Example 1. The data from the rest of 3 locations is given as below:

Yield in kg/ha

Strain No.	Sriganganagar		Navgaon			Hissar		
	Replications		Replications			Replications		
	1	2	1	2	3	1	2	3
1	778.00	667.00	533.28	488.84	799.92	945.68	1040.25	1040.25
2	556.00	444.00	444.40	488.84	466.62	567.41	945.68	803.83
3	556.00	444.00	977.68	888.80	799.92	1134.82	1182.10	1040.25
4	778.00	778.00	888.80	799.92	799.92	969.33	1229.39	1134.82
5	556.00	556.00	666.60	666.60	444.40	898.40	851.11	969.33
6	444.00	444.00	799.92	533.28	577.72	851.11	756.55	969.33
7	556.00	333.00	1066.56	1022.12	933.24	1134.82	1323.96	1040.25
8	556.00	444.00	1111.00	1066.56	1066.56	1229.39	1134.82	1134.82
9	444.00	556.00	666.60	888.80	844.36	1087.54	898.40	992.97
10	778.00	556.00	533.28	622.16	844.36	851.11	1134.82	945.68
11	667.00	778.00	1022.12	666.60	755.48	1040.25	1276.67	1229.39
12	444.00	444.00	799.92	666.60	622.16	803.83	945.68	992.97
13	333.00	556.00	799.92	666.60	688.82	992.97	1182.10	1323.96
14	444.00	333.00	888.80	933.24	666.60	1040.25	1134.82	1276.67
15	556.00	333.00	844.36	688.82	577.72	1182.10	1418.52	1229.39
16	333.00	333.00	711.04	622.16	622.16	1087.54	945.68	1040.25
17	556.00	333.00	799.92	577.72	533.28	969.33	1040.25	1040.25
18	333.00	333.00	1066.56	1111.00	999.90	969.33	1087.54	1040.25
19	444.00	444.00	933.24	711.04	711.04	1418.52	1040.25	945.68
20	444.00	444.00	755.48	799.92	733.26	1182.10	1134.82	1087.54
21	333.00	444.00	844.36	755.48	666.60	1087.54	1323.96	1040.25
22	444.00	333.00	666.60	533.28	488.84	992.97	803.83	992.97
23	556.00	333.00	755.48	799.92	1022.12	1134.82	992.97	1229.39
24	333.00	333.00	488.84	577.72	666.60	1040.25	992.97	1182.10

The data from each of the centers were analyzed separately using PROC GLM of SAS. The results for Bhatinda center are given in Example 1. The results of the other 3 locations are given in the sequel.

ANOVA (Yield: Hissar)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	1007589.069	43808.220	3.06	*0.0006*
Due to Replications	2	37465.039	18732.519	1.31	*0.2795*
Error	46	657493.58	14293.33		
Total	71	1702547.68			

R-Square	CV	Root MSE	Mean Yield
0.613818	11.30376	119.5548	1057.65

Treatments are significantly different at 5% level of significance, where as replications are not significantly different. None of the entries gave significantly higher yield than best performing check (Swarna Jyoti).

ANOVA (Yield: Navgaon)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	1685581.90	73286.19	6.51	*<0.0001*
Due to Replications	2	73332.38	36666.19	3.26	*0.0476*
Error	46	518154.24	11264.23		
Total	71	2277068.52			

R-Square	CV	Root MSE	Mean Yield
0.772447	14.15831	106.1330	749.6164

Both treatments and replications are significantly different at 5% level of significance. New entry at serial number 8 gave significantly higher yield than best performing check (Swarna Jyoti).

ANOVA (Yield: Sriganganagar)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	699720.92	30422.65	4.03	*0.0007*
Due to Replications	1	31314.08	31314.08	4.15	*0.0533*
Error	23	173540.92	7545.26		
Total	47	904575.92			

R-Square	CV	Root MSE	Mean Yield
0.808152	17.95781	86.86344	483.7083

Both treatments and replications are significantly different at 5% level of significance, New entry at serial number 8 gave significantly higher yield than best performing check (Swarna Jyoti). Error mean squares and error degrees of freedom of the 4 locations are:

	Bhatinda	Hissar	Navgaon	Sriganganagar
Error degrees of freedom	46	46	46	23
Error Mean Square	10067.91	14293.33	11264.23	7545.26

In order to perform the combined analysis of the data for 4 locations (group of experiments), the mean square errors for the 4 locations were tested for the homogeneity of error variances using Bartlett's χ^2-test. The test is described in the sequel:

Let the experiment is conducted in *k* environments. The estimate of error variance for the i^{th} environment is s_i^2 (MSE for i^{th} environment) with f_i degrees of freedom (error degrees of freedom).

We are interested to test the null hypothesis $H_0 : \sigma_1^2 = \sigma_2^2 = ... = \sigma_k^2$ against the alternative hypothesis H_1 : at least two of the σ_i^2'*s* are not equal, where σ_i^2 is the error variance for treatment *i*. (σ_i^2 is the error variance for the i^{th} environment).

The procedure involves computing a statistic whose sampling distribution is closely approximated by the χ^2 distribution with *k - 1* degrees of freedom. The test statistic is

$$\chi_0^2 = 2.3026 \frac{q}{c}$$

and null hypothesis is rejected when $\chi_0^2 > \chi_{\alpha,k-1}^2$, where $\chi_{\alpha,k-1}^2$ is the upper α percentage point of χ^2 distribution with *k - 1* degrees of freedom.

To compute χ_0^2, follow the steps:

Step 1: Compute mean and variance of all *v*-samples.

Step 2: Obtain pooled variance $S_p^2 = \dfrac{\sum_{i=1}^{k} f_i s_i^2}{\sum_{i=1}^{k} f_i}$

Step 3: Compute $q = \left(\sum_{i=1}^{k} f_i \right) \log_{10} S_p^2 - \sum_{i=1}^{k} f_i \log_{10} S_i^2$

Step 4: Compute $c = 1 + \dfrac{1}{3(k-1)} \left(\sum_{i=1}^{k} f_i^{-1} - \left(\sum_{i=1}^{k} f_i \right)^{-1} \right)$

Step 5: Compute χ_0^2.

For this example, the computed χ_0^2 was found to be 3.28. The tabulated value of $\chi_{3,0.05}^2 = 7.81$. Therefore, the null hypothesis is not rejected. Therefore, the error variances were found to be homogeneous. Now the combined analysis of data can be carried out using the following statements of SAS.

```
Data comb;
Input loc $ rep var yield;
Cards;
.
.
.
;
```

```
proc glm;
class loc rep trt;
model yield = loc rep(loc) trt trt*loc;
random loc rep(loc) trt*loc/test;
run;
```

The results obtained are:

Combined Analysis of Data Over 4 Locations of Rapeseed-Mustard Initial Varietal Trial

Source	DF	SS	Mean Square	F Value	Pr>F
loc	3	16794186.86	5598062.29	497.31	<.0001
Replications(loc)	7	298250.83	42607.26	3.79	0.0008
Treatments	23	2153545.49	93632.41	8.32	<.0001
loc*Treatment	69	3495630.98	50661.32	4.50	<.0001
Error	161	1812312.49	11256.60		
Total	263	24811785.12			

R-square	C.V.	Root MSE	Mean
0.93	11.81	106.10	898.59

Source	Type III Expected Mean Square
loc	Var(Error) + 2.7273 Var(loc*treatment) + 24 Var(rep(loc) + 65.455 Var(loc)
rep(loc)	Var(Error) + 24 Var(rep(loc)
treatment	Var(Error) + 2.6667 Var(loc*treatment) + Q(treatment)
loc*treatment	Var(Error) + 2.7273 Var(loc*treatment)

Tests of Hypotheses for Mixed Model Analysis of Variance

Source	DF	SS	MS	F-Value	Pr>F
loc	3	16794187	5598062	68.26	<.0001
*Error: MS(rep(loc)) + MS(loc*treatment) - MS(Error)*					
rep(loc)	7	298251	42607	3.79	0.0008
loc*treatment	69	3495631	50661	4.50	<.0001
Error: MS(Error)					
treatment	23	2153545	93632	1.88	0.0232
*Error: 0.9778*MS(loc*treatment)+0.0222*MS(Error)*					

Example 6.2: An experimenter was interested in comparing *49* treatments. The experiment was laid out in a lattice design with four replications. There were seven blocks per replication and seven treatments were allotted within each block. Observations were recorded on several characters but for illustration purposes only one data set (one character) is analyzed. The same design was repeated over two years. The layout of the design is given below:

Blocks			**Replication - I**				
1.	*1*	*2*	*3*	*4*	*5*	*6*	*7*
2.	*8*	*9*	*10*	*11*	*12*	*13*	*14*
3.	*15*	*16*	*17*	*18*	*19*	*20*	*21*
4.	*22*	*23*	*24*	*25*	*26*	*27*	*28*
5.	*29*	*30*	*31*	*32*	*33*	*34*	*35*
6.	*36*	*37*	*38*	*39*	*40*	*41*	*42*
7.	*43*	*44*	*45*	*46*	*47*	*48*	*49*
Blocks			**Replication - II**				
1.	*1*	*8*	*15*	*22*	*29*	*36*	*43*
2.	*2*	*9*	*16*	*23*	*30*	*37*	*44*
3.	*3*	*10*	*17*	*24*	*31*	*38*	*45*
4.	*4*	*11*	*18*	*25*	*32*	*39*	*46*
5.	*5*	*12*	*19*	*26*	*33*	*40*	*47*
6.	*6*	*13*	*20*	*27*	*34*	*41*	*48*
7.	*7*	*14*	*21*	*28*	*35*	*42*	*49*
Blocks			**Replication - III**				
1.	*1*	*9*	*17*	*25*	*33*	*41*	*49*
2.	*43*	*2*	*10*	*18*	*26*	*34*	*42*
3.	*36*	*44*	*3*	*11*	*19*	*27*	*35*
4.	*29*	*37*	*45*	*4*	*12*	*20*	*28*
5.	*22*	*30*	*38*	*46*	*5*	*13*	*21*
6.	*15*	*23*	*31*	*39*	*47*	*6*	*14*
7.	*8*	*16*	*24*	*32*	*40*	*48*	*7*
Blocks			**Replication - IV**				
1.	*1*	*37*	*24*	*11*	*47*	*34*	*21*
2.	*15*	*2*	*38*	*25*	*12*	*48*	*35*
3.	*29*	*16*	*3*	*39*	*26*	*13*	*49*
4.	*43*	*30*	*17*	*4*	*40*	*27*	*14*
5.	*8*	*44*	*31*	*18*	*5*	*41*	*28*
6.	*22*	*9*	*45*	*32*	*19*	*6*	*42*
7.	*36*	*23*	*10*	*46*	*33*	*20*	*7*

The analysis was carried out using **PROC GLM** of **SAS** and using the option of contrast for carrying out the contrast analysis. The results of the analysis of data for the first year are as given below:

RESULTS 1 (LATTICE DESIGN: FIRST YEAR)

Source	DF	SS	Mean Square	F Value	Pr>F
Replications	3	186.04	62.01	7.53	0.0001
Block(replication)	24	358.94	14.95	1.82	0.0192
Treatments	48	3442.14	71.71	8.70	0.0001
Error	120	988.70	8.23		
Total	195	6025.75			

R-square	C.V.	Root MSE	Mean
0.84	3.37	2.87	85.18

It may be noted that all sum of squares reported in the table are adjusted sums of squares and that the adjustments have been made for all the other remaining effects. The CV is very small and, therefore, the design adopted is appropriate. The interesting feature of the design is that the blocks within replication sum of squares are significant and, therefore, formation of blocks within replications has been fruitful. Thus, the formation of incomplete blocks within replications has been very effective and the error mean square is quite small. The treatment effects are also highly significant. The *49* treatments tried in the experiment were formed into four groups on the basis of the nature of the treatments. The groups are - Group *1*: Treatments *1 - 15*; Group *2*: Treatments *16 - 30*; Group *3*: Treatments *31 - 46*; Group *4*: Treatments *47 - 49*. Contrast analysis was carried out to study the equality of the treatment effects within groups and desired between group comparisons. The results are as follows:

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
gr1	14	985.53	70.39	8.54	0.0001
gr2	14	1004.60	71.75	8.71	0.0001
gr3	15	1373.17	91.54	11.11	0.0001
gr4	2	60.27	30.13	3.66	0.0287
gr1 vs gr4	1	47.29	47.29	5.74	0.0181
gr2 vs gr4	1	92.69	92.69	11.25	0.0011
gr3 vs gr4	1	41.74	41.74	5.07	0.0262
gr1 vs gr2	1	18.86	18.86	2.29	0.1329

It may be seen that the group *1* vs group *2* comparisons are not significantly different whereas all other comparisons are significantly different.

RESULT 2 (LATTICE DESIGN SECOND YEAR)

Source	DF	SS	Mean Square	F Value	Pr>F
Replications	3	176.404	58.79	11.81	0.0001
Block(replication)	24	556.49	23.18	4.66	0.0001
Treatments	48	3353.21	69.85	14.03	0.0001
Error	120	597.30	4.97		
Total	195	5413.92			

R-square	C.V.	Root MSE	Mean
0.89	2.50	2.23	89.31

It may be noted again that all sum of squares reported in the table are adjusted sums of squares and that the adjustments have been made for all other remaining effects. The CV is very small and therefore the design adopted is appropriate. The interesting feature of the design is that the blocks within replication sum of squares are highly significant and, therefore, formation of blocks within replications has been fruitful. Thus, the formation of incomplete blocks within replications has been very effective and the error mean square is quite small. The treatment effects are also highly significant.

In order to perform the combined analysis of the data for two years (group of experiments), the mean square errors for the two years were tested for the homogeneity of error variances. The value of *F* statistic was obtained as *F = MSE1 / MSE2 = 8.23 / 4.97 = 1.6559* (significant at *5* % level of significance). Therefore, for performing the combined analysis weighted least squares was done, the weight being the reciprocals of the root mean square error. The weighted least squares analysis is carried out by defining a new variable *newres = res/root mean square error*. The analysis of variance is then performed on the new variable. The following analysis is usually carried out for these situations.

RESULT 3 (LATTICE DESIGN COMBINED ANALYSIS FOR YEARS 1 & 2)

Source	DF	SS	Mean Square	F Value	Pr>F
Year	1	4911.42	4911.42	3672.45	0.0001
Replications	3	19.27	6.42	4.80	0.0028
Block(replication)	24	93.56	3.90	2.91	0.0001
Treatments	48	1142.94	23.81	17.80	0.0001
Year*Treatment	48	137.75	2.87	2.15	0.0001
Error	267	357.08	1.34		
Total	391	6780.42			

R-square	C.V.	Root MSE	Mean
0.95	3.34	1.16	34.66

The year*treatment interaction is highly significant. Therefore, treatment is tested against the year*treatment interaction mean square. The results obtained are given as:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	48	1142.94	23.81	8.30	<.0001

In the above analysis, the degrees of freedom for the replications and blocks (replications) are 3 and 24 respectively and are same as that of individual year analyses. Therefore, no distinction is made in the replications and blocks (replications) of the two years. Hence, this procedure is inappropriate.

The appropriate procedure, therefore, is to view the groups of experiments as a nested design with several factors nested within one another. The locations are treated as big blocks, with the experiments nested within these. The combined analysis of data, therefore, can be done as that of a nested design. An advantage of this analysis is that there is a further reduction in the error sum of squares because one more source of variability is

taken out from the experimental error thus reducing the experimental error. This may also lead to the reduction in the value of CV. If we take the data for two years together there will be *56* blocks and hence the blocks will account for *55* degrees of freedom. The analysis of variance just described accounts for 28 degrees of freedom. The remaining *27* degrees of freedom go into the error. However, if we analyze the data as a nested design, we get *55* degrees of freedom for the blocks that can be split into various components. In the sequel, we present the appropriate analysis of groups of experiments. This enables us to further reduce the experimental error thus reducing the CV. The results obtained are reproduced below:

RESULT 4. (LATTICE DESIGN COMBINED ANALYSIS CONSIDERING NESTED CLASSIFICATIONS - REPLICATIONS NESTED WITHIN YEARS AND BLOCKS NESTED WITHIN REPLICATIONS AND YEARS ON THE TRANSFORMED DATA)

Source	DF	SS	Mean Square	F Value	Pr>F
Year	1	4911.42	4911.42	4344.23	<.0001
Replications(Year)	6	58.83	9.80	8.67	<.0001
Blocks(Year*replication)	48	139.74	2.91	2.58	<.0001
Treatments	48	968.42	20.18	17.85	<.0001
Year*Treatment	48	130.86	2.73	2.41	<.0001
Error	240	271.33	1.13		
Total	391	6780.42			

R-square	C.V.	Root MSE	Mean
0.96	3.07	1.06	34.66

It may be seen that the error sum of squares has a reduction of *27* degrees of freedom. The CV has also reduced from *3.34* to *3.07*. The sums of squares due to various components in the model are highly significant. The advantage of analyzing the data as a nested design is quite visible thus.

The year*treatment interaction is highly significant. Therefore, treatment is tested against the year*treatment interaction mean square. The results obtained are given as:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	48	968.42	20.18	7.40	<.0001

In the above analysis, the proper error terms can also be identified using PROC GLM of SAS along with random statement with TEST option. Using PROC GLM, the expected mean squares for different effects in the model are given as

Source	Type III Expected Mean Square
Year	Var(Error) + 4 Var(year*treatment) + 7 Var(block(year*rep)) + 49 Var(rep(year)) + 196 Var(year)
rep(year)	Var(Error) + 7 Var(block(year*rep)) + 49 Var(rep(year))
block(year*rep)	Var(Error) + 5.25 Var(block(year*rep))
treatment	Var(Error) + 3.5 Var(year*treatment) + Q(treatment)
year*treatment	Var(Error) + 3.5 Var(year*treatment)

Tests of Hypotheses for Mixed Model Analysis of Variance

Source	DF	SS	MS	F-Value	Pr>F
Year	1	4911.42	4911.42	422.37	<.0001
*Error: MS(rep(year)) + 1.1429*MS(year*treatment) - 1.1429*MS(Error)*					
rep(year)	6	58.83	9.80	2.80	0.0232
*Error: 1.3333*MS(block(year*rep)) - 0.3333*MS(Error)*					
block(year*rep)	48	139.74	2.91	2.58	<.0001
year*treatment	48	130.86	2.73	2.41	<.0001
Error: MS(Error)					
treatment	48	968.42	20.18	7.40	<.0001
*Error: MS(year*treatment)*					

It is observed that the year*treatment interaction is highly significant and the proper error term for testing the equality of treatment effects is year*treatment interaction mean square.

The above discussions refer to the combined analysis of experiments conducted at different locations or different times at the same location in general block designs with same treatments in each of the environments. There may arise situations where all the treatments are not common to the whole set. Only subsets of the treatments are common to the whole set. This may happen due to some location specific treatments that cannot be tried at all the locations. Different treatments at different locations do not give any problem in the combined analysis of data so long as there are some common treatments over all the locations.

7. Factorial Experiments

Example 7.1: An experiment was conducted at Ludhiana Centre of AICRP on Cropping Systems using a balanced confounded design for factorial experiments with three factors, *viz.*, Nitrogen (40, 80 and 120 kg/ha), Phosphorous (0, 40 and 80 kg/ha) and Potassium (0 and 40 kg/ha). These *18* treatment combinations were arranged in *3* blocks of size *6* each.

The analysis of the data was performed using PROC GLM of SAS. The SAS commands and the output is given in the sequel.

```
Options linesize=72;
data ludh98k;
input rep     block     N     P     K     trt     yield;
cards;
```

1	1	40	0	0	1	7.79
1	1	120	80	0	17	10.30
1	1	40	80	40	6	10.08
1	1	120	40	40	16	11.66
1	1	80	0	40	8	9.13
1	1	80	40	0	9	10.56
1	2	40	0	40	2	6.12
1	2	120	0	0	13	8.44
1	2	120	80	40	18	11.44
1	2	80	40	40	10	9.13
1	2	80	80	0	11	9.40
1	2	40	40	0	3	6.85
1	3	80	0	0	7	6.25
1	3	120	0	40	14	7.78
1	3	40	40	40	4	6.66
1	3	80	80	40	12	9.42
1	3	40	80	0	5	6.50
1	3	120	40	0	15	11.82
2	1	120	0	0	13	7.86
2	1	120	40	40	16	10.15
2	1	40	80	40	6	7.50
2	1	80	0	40	8	7.89
2	1	80	80	0	11	8.00
2	1	40	40	0	3	6.40
2	2	120	0	40	14	8.50
2	2	80	80	40	12	9.86
2	2	40	40	40	4	7.70
2	2	120	80	0	17	10.79
2	2	80	40	0	9	7.87
2	2	40	0	0	1	6.30
2	3	80	0	0	7	7.00
2	3	40	80	0	5	8.00
2	3	120	80	40	18	10.90
2	3	40	0	40	2	6.62
2	3	80	40	40	10	9.62
2	3	120	40	0	15	9.50
3	1	80	80	0	11	10.00
3	1	120	80	40	18	10.86
3	1	40	40	40	4	7.58
3	1	80	0	40	8	6.35
3	1	120	40	0	15	9.40
3	1	40	0	0	1	5.94

```
3 2 120 0 40 14 9.00
3 2 40 80 40 6 8.80
3 2 80 40 40 10 9.53
3 2 120 80 0 17 10.56
3 2 40 40 0 3 7.07
3 2 80 0 0 7 6.00
3 3 80 40 0 9 7.20
3 3 120 0 0 13 8.36
3 3 40 0 40 2 6.05
3 3 80 80 40 12 10.45
3 3 120 40 40 16 10.10
3 3 40 80 0 5 7.50
4 1 80 80 0 11 7.97
4 1 80 40 40 10 7.18
4 1 40 80 40 6 6.16
4 1 40 0 0 1 4.95
4 1 120 40 0 15 10.12
4 1 120 0 40 14 7.15
4 2 80 0 0 7 6.65
4 2 40 40 0 3 6.66
4 2 80 80 40 12 7.90
4 2 120 40 40 16 10.10
4 2 40 0 40 2 6.49
4 2 120 80 0 17 10.30
4 3 80 0 40 8 6.12
4 3 40 40 40 4 5.80
4 3 120 80 40 18 10.06
4 3 120 0 0 13 7.37
4 3 80 40 0 9 7.24
4 3 40 80 0 5 7.70
;
proc glm;
class rep block n p k;
model yield = rep block(rep)  n p k n*p n*k p*k n*p*k;
run;
```

The output is given as:

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F-Vlaue	Pr>F
Model	28	185.8448	6.6373	13.56	<0.0001
Error	43	21.0427	0.4894		
Corrected Total	**71**	206.8876			

R-Square	Coeff Var	Root MSE	Yield Mean
0.8983	8.4444	0.699547	8.284

Source	DF	Type III SS	Mean Square	F-Vlaue	Pr>F
Rep	3	15.7187	5.2396	10.71	<.0001
Block(rep)	8	14.1946	1.7743	3.63	0.0027
N	2	89.1108	44.5554	91.05	<.0001
P	2	55.9270	27.9635	57.14	<.0001
K	1	3.2173	3.2173	6.57	0.0139
NP	4	4.2752	1.0688	2.18	0.0868
NK	2	0.7301	0.3650	0.75	0.4803
PK	2	0.1128	0.0564	0.12	0.8914
NPK	4	2.1958	0.5490	1.12	0.3588

From the above, it is clear that the blocks with in replication are significant indicating that the incomplete blocks have help in reducing mean square error. All the three main effects N, P and K are significant whereas none of the interaction is significant.

References

Kempthorne, O. (1977). Why randomize? *Journal of Statistical Planning and Inference*, **1**, 1-25.

Dean, A. and Voss, D. (1999). *Design and Analysis of Experiments*. Springer Text in Statistics, New York.

Fisher, R.A. and Yates, F. (1963). *Statistical Tables For Biological, Agricultural and Medical Research*. Longman Group Ltd., England.

Exercise 1.1: In order to select suitable tree species for Fuel, Fodder and Timber an experiment was conducted in a randomized complete block design with ten different trees and four replications. The plant height was recorded in cms. The details of the experiment are given below:

Plant Height (Cms): Place – Kanpur

Name of Tree	Spacing	Replications			
		1	2	3	4
A. Indica	4x4	144.44	145.11	104.00	105.44
D. Sisso	4x2	113.50	118.61	118.61	123.00
A. Procer	4x2	60.88	90.94	80.33	92.00
A. Nilotic	4x2	163.44	158.55	158.88	153.11
T. Arjuna	4x2	110.11	116.00	119.66	103.22
L. Loucoc	4x1	260.05	102.27	256.22	217.80
M. Alba	4x2	114.00	115.16	114.88	106.33
C. Siamia	4x2	91.94	58.16	76.83	79.50
E. Hybrid	4x1	156.11	177.97	148.22	183.17
A. Catech	4x2	80.2	108.05	45.18	79.55

Analyze the data and draw your conclusions.

Procedure and Calculations: We compute the following totals:

Treatments totals ($y_{i.}$)	**Treatment means ($\bar{y}_{i.} = y_{i.} / b$)**
$y_{1.}$ = 144.44 +…+ 105.44 = 498.99	$\bar{y}_{1.}$ = 498.99/4 = 124.7475
$y_{2.}$ = 112.50 + … + 123.00 = 473.72	$\bar{y}_{2.}$ = 473.72/4 = 118.4300
$y_{3.}$ = 60.88 + … + 92.00 = 324.15	$\bar{y}_{3.}$ = 324.15/4 = 81.0375
$y_{4.}$ = 163.44 + … + 153.11 = 633.98	$\bar{y}_{4.}$ = 633.98/4 = 158.4950
$y_{5.}$ = 110.11 + … + 103.22 = 448.99	$\bar{y}_{5.}$ = 448.99/4 = 112.2475
$y_{6.}$ = 260.05 + … +217.8 = 836.34	$\bar{y}_{6.}$ = 836.34/4 = 209.0850
$y_{7.}$ = 114.00 + … + 106.33 = 450.37	$\bar{y}_{7.}$ = 450.37/4 = 112.5925
$y_{8.}$ = 91.94 + … + 79.50 = 306.43	$\bar{y}_{8.}$ = 306.43/4 = 76.6075
$y_{9.}$ = 156.11 + … + 183.17 = 665.47	$\bar{y}_{9.}$ = 665.47/4 = 166.3675
$y_{10.}$ = 80.20 + … + 79.55 = 312.98	$\bar{y}_{10.}$ = 312.98/4 = 78.2450

Replication (or Blocks) Totals ($y_{.j}$)	**Replication Means ($\bar{y}_{.j} = y_{.j} / v$)**
$y_{.1}$ = 144.44 + … + 80.20 = 1294.67	$\bar{y}_{.1}$ = 1294.67/10 = 129.4670
$y_{.2}$ = 145.11 + … + 108.05 = 1190.82	$\bar{y}_{.2}$ = 1190.82/10 = 119.0820
$y_{.3}$ = 104.00 + … + 45.18 = 1222.81	$\bar{y}_{.3}$ = 1222.81/10 = 122.2810
$y_{.4}$ = 105.44 + … + 79.55 = 1243.12	$\bar{y}_{.4}$ = 1243.12/10 = 124.3120

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 4951.42$.

Correction Factor = $(y_{..})^2 / vb = (4951.42)^2/40 = 612914.0004$

Sum of Squares due to Trees = $\sum_i y_{i.}^2 / b - C.F.$

$$= \left(498.99^2 + \cdots + 312.98^2\right)/ 4 - 612914.00 = 66836.35$$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$$= \left(1294.67^2 + \cdots + 1243.12^2\right)/ 10 - 6121914.00 = 569.43 .$$

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$$= 144.44^2 + 145.12^2 + \cdots + 79.55^2 - C.F. = 89101.42 .$$

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to Trees - Sum of

Squares due to Replications = *89101.42 - 66836.35 - 569.43 = 21695.26.*

We now form the following Analysis of Variance Table:

ANOVA

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Trees	*9*	*66836.35*	*7426.26*	*9.24*	*0.0001*
Due to Replications	*3*	*569.43*	*189.81*	*0.24*	*0.8703*
Error	*27*	*21695.26*	*803.53*		
Total	*39*	*89101.04*			

Critical Difference between any two tree means = $t_{\alpha, error\ d.f.} x\sqrt{2MSE / b}$

$$= 2.05x\sqrt{(2x803.53)/ 4} = 41.09$$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two trees effects:

					Mean	Tree No.
				A	*209.085*	*6*
			B		*166.368*	*9*
		C	*B*		*158.500*	*4*
	D	*C*			*124.748*	*1*
E	*D*	*C*			*118.430*	*2*

F	*E*	*D*	*112.593*	*7*
F	*E*	*D*	*112.248*	*5*
F	*E*		*81.038*	*3*
F	*E*		*78.245*	*10*
F			*76.608*	*8*

Suppose now that tree numbers *1, 2, 3, 4, 10* and trees numbers *5, 6, 7, 8, 9* form two groups on the basis of some considerations. (The first group of trees is useful for fuel, fodder and timber while the second group of trees is useful for fuel and fodder only). Our interest is in comparing the two groups. We shall have the following contrast to be estimated and tested:

1. $t_1 + t_2 + t_3 + t_4 - t_5 - t_6 - t_7 - t_8 - t_9 + t_{10}$.

Similarly, suppose the other contrasts to be estimated and tested are:

2. $9t_1 - t_2 - t_3 - t_4 - t_5 - t_6 - t_7 - t_8 - t_9 - t_{10}$
3. $t_1 + t_2 + t_3 + t_4 - 4t_9$
4. $t_1 + t_2 + t_3 + t_4 - 4t_{10}$
5. $t_5 + t_6 + t_7 + t_8 - 4t_9$
6. $t_5 + t_6 + t_7 + t_8 - 4t_{10}$
7. $t_9 - t_{10}$

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
1	*1*	*788.3285*	*788.3285*	*0.98*	*0.3307*
2	*1*	*4.1131*	*4.1131*	*0.01*	*0.9435*
3	*1*	*6680.2435*	*6680.2435*	*8.31*	*0.0076*
4	*1*	*5761.6546*	*5761.6546*	*7.17*	*0.0125*
5	*1*	*4801.1258*	*4801.1258*	*5.98*	*0.0213*
6	*1*	*7805.3981*	*7805.3981*	*9.71*	*0.0043*
7	*1*	*15531.1500*	*15531.1500*	*19.33*	*0.0002*

Suppose now that the interest of the experimenter is to test certain hypothesis concerning the five trees in the Group 1 (comprising of Trees Numbers *1, 2, 3, 4,* and *10*). The sum of squares for testing the equality of tree effects can be obtained by defining four mutually orthogonal contrasts as $t_1 - t_2$; $t_1 + t_2 - 2t_3$; $t_1 + t_2 + t_3 - 3t_3$; $t_1 + t_2 + t_3 + t_4 - 4t_{10}$. Using these sets of contrasts we get the following:

Sl. No.	D.F.	S.S.	M.S.	F	Pr > F
1	4	17854.0908	4463.5227	5.55	0.0021