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Experimental Design and Analysis L2.

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Multiple comparison tests

The most important multiple comparison tests are: -

1. **least significant difference test [LSD α]** which can be use in case of : -

A - Equal replicates :

$$\begin{aligned} \text{LSD}\alpha &= \text{tab. } t\alpha * \sqrt{2\text{MSE}/r} \\ &= \sqrt{2} \text{ tab. } t\alpha * \sqrt{\text{MSE}/r} \\ &= 1.414 \text{ tab. } t\alpha * \overline{Sx} \end{aligned}$$

B - Un equal replicates:

$$\text{LSD}\alpha = \text{tab. } t\alpha * \sqrt{2\text{MSE}/K_0}$$

$$K_0 = 1/ t-1 [\sum ri - \sum ri^2 / \sum ri] \quad \text{or}$$

$$K_0 = \sum ri / t$$

t = number of treatment.

C - Revised least significance difference [RLSD α] : -

$$\text{RLSD} = \text{tab. } t_{\alpha} * \sqrt{2\text{MSE}/r}$$

Tab. t_{α} = Revised tab. t_{α} which depends on: -

1-df of treat. 2-df of Error. 3-Calculated F. 4-Level of significance.

For the above reason RLSD α is more accurate.

D-Protected LSD.

E-Non-protected LSD

F- Least significant increase test (LSI): In many plant breeding - trails new varieties are compared with standard varieties

The purpose to find new cultivar that give higher yields or quality than the standards. You lose interest if a new variety gives a significantly lower result. A criterion that can be used to test yielding whether each new variety is significantly higher than a standard is the **LSI** procedure which is the one-sided version of LSD test.

It consists of a series of one tailed t-tests.

$LSI = \text{tab. } t_{\infty} * \sqrt{2MSE/r}$ or $LSI = \text{tab. } t_{\infty} * SED$ where

$SED =$ Standard error of the difference between two means.

Any variety with a mean yield greater than(mean + LSI) significantly out yields the standard.

Steps for using LSD test :

1. Calculating $LSD\alpha$ value.
2. Calculating means of treatments.
3. Arranging means of treatments vertically from the highest to the lowest value.
4. Arranging means of treatments horizontally from the lowest value to the highest value.
5. Calculating all possible differences between the mean of treatments then comparing the results with calculated $LSD\alpha$ if the difference between the mean of two treatment is **equal** or **more** than calculated $LSD\alpha$ it means there is a **significance** difference between them, but if the difference between two means is **less** than $LSD\alpha$ it means there is **no significance** difference between them.

Homework: According the following information complete the ANOVA table then compare between means if:

1- No. of exp. units=25 , $SS_{\text{Error}}=0.90$, C.F.=200

2-No. of treatment=5 , sum of t_1 , t_2 , t_4 and t_5 were 13,15,17 and 8 respectively ,

2. Duncan's multiple range test: -

$$\text{LSR} = \text{SSR} * \sqrt{\text{MSE}/r}$$

LSR = least significant range.

SSR = Sufficient student's range values [table values].

The number of table SSR values are equal to (**number of treatments-1**) in this test the maximum difference between means must be compare with maximum LSR value and the minimum value of difference between treatments must be compare with the lowest LSR value.

Steps for using Duncan's test: -

It is similar to the steps used in LSD α in most steps expect the following step: -

The LSR values must be arrange vertically from the highest to the lowest value and beside the means, then using special technique for comparison in order to comparing the highest difference between means with the highest LSR value and the lowest difference with lowest LSR ...

3. Dunnett's test:

This test can be use for comparing between the mean of treatments and the mean of the control treatment only.

$$Dt = tab Dt(\alpha, dft, dfE) * \sqrt{2MSE/r}$$

4. Tukey's Honestly significant difference test (HSD) also called Tukey's Studentised Range Test.

Turkeys test or method:

$$\bar{Y}_i - \bar{Y}_j \pm Q_{0.05/\sqrt{2}} * \sqrt{2MSE/r}$$

$\bar{Y}_i - \bar{Y}_j \pm Q_{0.05} * \sqrt{MSE/r}$ if the range contains zero value it means there is no significant difference between two means of treatments ,but if the range not contains zero value it means there is significant difference between them.

MSD=Q value * $\sqrt{MSE/r}$ MSD=Minimum significant difference. Q= table value.

Increase in number of treatments causes increase in table value.

5. Student-Newman-keuls Test (SNK).

Multiple comparison test

From the following information compare between treatments

<u>Treatments</u>	<u>sum</u>	<u>means</u>		
t1	74	74/5=14.8	MS error =2.50	r =5
t2	84	84/5=16.8		
t3	82	82/5=16.4	Tab. $t_{0.01} = 2.921$	
t4	103	103/5=20.6		

Means	$\bar{t}_1 = 14.8$	$\bar{t}_3 = 16.4$	$\bar{t}_2 = 16.8$	$\bar{t}_4 = 20.6$
$\bar{t}_4 = 20.6$	$t_4 - t_1 = 20.6 - 14.8 = 5.8^{**}$	$20.6 - 16.4 = 4.2^{**}$	$20.6 - 16.8 = 3.8^{**}$	0
$\bar{t}_2 = 16.8$	$16.8 - 14.8 = 2.0^{n.s.}$	$16.8 - 16.4 = 0.4^{n.s.}$	0	
$\bar{t}_3 = 16.4$	$16.4 - 14.8 = 1.6^{n.s.}$	0		
$\bar{t}_1 = 14.8$	0			

$$1 - LSD_{\alpha} = tab.t_{\alpha, df_{error}} \sqrt{\frac{2MSE}{r}} \quad or = tab.t_{\alpha, df_{error}} \sqrt{2} S_{\bar{x}}$$

$$LSD_{0.01} = tab.t_{(0.01, 16)} \sqrt{\frac{2(2.5)}{5}} = 2.921\sqrt{1} = \mathbf{2.921}$$

There are significant differences between (t1 and t4), (t3 and t4) and (t2 and t4)
but no significant differences between (t1 and t2), (t3 and t2) & (t1 and t3).

$$2 - RLSD_{\alpha} = tab.t' \sqrt{\frac{2MSE}{r}}$$

$$RLSD_{0.01} = tab.t' \sqrt{\frac{2(2.5)}{5}} = (2.66)\sqrt{1} = \mathbf{2.66}$$

There are significant differences between (t1 and t4), (t3 and t4), (t2 and t4)
but no significant differences between (t1 and t2), (t3 and t2) & (t1 and t3).

Also we can use DMRT and Dunnett's test.

Exp.1/ A study was conducted to compare between (5) species of rhizobium depending on number of active nodules/plant ,if the number of pots used in experiment were [21]pots and you are given the following information. Compare between treatments using LSD.01

Species	r 1	r2	r3	r4	r5	sum
T1	20	18	19	20	17	94
T2	21	22		21		64
T3	23	24	25	25	24	121
T4	26	26	25	25		162
T5	29	27	25	26		107

$$C.F. = (388)^2 / 21 = 7168.76$$

$$SST = (20)^2 + \dots + (26)^2 - 7168.76 = 203.82SS$$

$$SSE = SS \text{ Total} - SS \text{ treat}$$

$$SS \text{ treat} = ([94]^2/5 + [66]^2/3 + \dots + [107]^2/4) - 7168.76 = 183.8$$

$$= 203.82 - 183.8 = 20.02S$$

S.O.V	df	SS	MSE	Calc.F	Tab.F
Treat	$t-1=4$	183.3	45.8	37.89	
Error	$\sum r_i - t=16$	20.03	1.25		
Total	$\sum r_i - 1=20$				

Exp.2/ A pot experiment was conducted in glass house for testing the infection of 4 varieties of wheat with black stem rust disease, does there is any significant difference between varieties? Complete ANOVA table then compare between them using $LSD_{0.05}$, $RLSD_{0.05}$ and DMRT. Then mention your comments.

Treat.	r1	r2	r3	r4	$\sum t_i$	means
A	9	8	9	10	36	9
B	8	7	7	9	31	7.75
C	7	5	6	6	24	6
D	9	12	8	13	42	10.5
					G=133	

$$C.F. = (133)^2 / 4 * 4 = 1105.56$$

$$\sum x_{ij}^2 = (9)^2 + (8)^2 + \dots + (13)^2 = 1173$$

$$SST = \sum x_{ij}^2 - CF = 1173 - 1105.56 = 67.44$$

$$SS_t = \frac{(36)^2 + (31)^2 + (24)^2 + (42)^2}{4} - 1105.56 = 1149.25 - 1105.56 = 43.7$$

$$SS_E = 67.44 - 43.7 = 23.74$$

$$MS_t = 43.7 / 3 = 14.57$$

$$MS_E = 23.74 / 12 = 1.98$$

$$Cal.F = 14.57 / 1.98 = 7.36$$

Comment : the data must be analyze at level of significance .0.01 since it is a pot experiment:

S.O.V.	df	SS	MS	Tab.F _{0.05}	Cal.F
Treats	3	43.7	14.58	5.49	7.36**
Error	12	23.74	1.98		
Total	15	67.44			

\bar{t}_4	\bar{t}_1	\bar{t}_2	\bar{t}_3
10.5	9.0	7.75	6.0
a	ab	bc	c

$$\bar{t}_4 - \bar{t}_1 = 10.5 - 9.0 = 1.5^{n.s.}$$

$$\bar{t}_4 - \bar{t}_2 = 10.5 - 7.75 = 2.75^*$$

$$\bar{t}_4 - \bar{t}_3 = 10.5 - 6.0 = 4.5^*$$

$$\bar{t}_1 - \bar{t}_2 = 9.0 - 7.75 = 1.25^{n.s.}$$

$$\bar{t}_1 - \bar{t}_3 = 9.0 - 6.0 = 3.0^*$$

$$\bar{t}_2 - \bar{t}_3 = 7.75 - 6.0 = 1.75^{n.s.}$$

$$LSD_{\alpha} = tab.t_{\alpha, df_{error}} \sqrt{\frac{2MSE}{r}}$$

$$LSD_{0.05} = tab.t_{(0.05,12)} \sqrt{\frac{2(1.98)}{4}}$$

$$= 2.179(0.99) = \mathbf{2.16}$$

$$LSD'_{0.05} = tab.t'_{(0.05,3,12,7.36)} \sqrt{\frac{2(1.98)}{4}}$$

$$= 2.1(0.99) = \mathbf{2.079}$$

$$LSR = SSR\sqrt{MS_E / r}$$

$$S_{\bar{x}} = \sqrt{1.98/4} = 0.7$$

	2	3	4
SSR	3.08	3.23	3.33
sx or SE=0.7			
LSR	2.156	2.261	2.331

Means	LSR	\bar{t}_3	\bar{t}_2	\bar{t}_1	\bar{t}_4
$\bar{t}_4 = 10.5$	2.331	4.5*	2.75*	1.5 ^{n.s.}	0
$\bar{t}_1 = 9.0$	2.261	3.0*	1.25 ^{n.s.}	0	
$\bar{t}_2 = 7.75$	2.156	1.75 ^{n.s.}	0		
$\bar{t}_3 = 6.0$	-----	0			

There are significance differences between (t_4 and t_3), (t_4 and t_2) and (t_1 and t_3)

Example-3/ A study was conducted to compare between (5) types of cool drinks (no color, red, yellow, orange and green) by 25 persons ,if you are given the following data, construct ANOVA table at $\alpha = 0.01$ if you know that (t1 is control treatment).

Treat.	r1	r2	r3	r4	r5	sum	means
No color =t1	25.1	29.0	27.3	26.5	28.4	136.3	27.3
Red =t2	28.3	27.9	30.6	31.3	29.8	147.9	29.6
Yellow =t3	28.9	25.6	23.7	25.1	25.0	128.3	25.7
Orang=t4	25.4	28.5	26.9	24.6	27.1	132.5	26.5
Green =t5	31.8	29.6	29.5	32.0	31.7	154.6	30.9
						G=699.6	

$$C.F. = (699.6)^2 / 25 = 19577.6$$

$$\sum x_{ij}^2 = (25.1)^2 + (29)^2 + \dots + (31.7)^2 = 19722.9$$

$$SST = 19722.9 - 19577.6 = 145.3$$

$$SS_{treat.} = \frac{(136.3)^2 + \dots + (154.6)^2}{5} - 19577.6$$

$$= 19674.1 - 19577.6 = 96.5$$

$$SS_{Error} = 145.3 - 96.5 = 48.8$$

S.O.V.	df	SS	MS	Cal.F	Tab.F _{0.05}
Treatment	4	96.5	24.125	9.88*	2.87
Error	20	48.8	2.44		
Total	24	145.3			

While cal-F is more than tab-F we reject H₀ that's mean the treatments have significance effect . Donnets test:

$$\begin{aligned} \bar{t}_2 - \bar{t}_1 &= 29.6 - 27.3 = 2.3^{n.s.} & \bar{t}_3 - \bar{t}_1 &= 25.7 - 27.3 = -1.6^{n.s.} \\ \bar{t}_5 - \bar{t}_1 &= 30.9 - 27.3 = 3.6^* & \bar{t}_4 - \bar{t}_1 &= 26.5 - 27.3 = -0.8^{n.s.} \end{aligned}$$

$$D_t = tab.t_{D(\alpha, df_t, df_E)} \sqrt{2MS_E / r} = tab.t_{D(0.05, 4, 20)} \sqrt{2(2.44) / 5}$$

$$D_t = 2.7(0.98) = \mathbf{2.66}$$

There are differences between (t1) and (t5), (t5) is the best.

Q/ From the following data compare between levels of nitrogen (N) if the (t4) is control.

N levels.	r1	r2	r3	r4
N ₃₀	55	70	64	63
N ₂₀	58	59	67	63
N ₁₀	52	56	51	56
N ₀	49	50	46	52

- Example(Home work) :The study was conducted to test the effect of 6 levels of phosphorus fertilizer (0,1.5 ,3, 4.5 ,6 and 7.5 g/pot on weight of broad bean g/ pot. If you are given the following information :
- 1- $\sum t_1=150$, $\sum t_2 =180$, $\sum t_3 =210$, $\sum t_4 =240$, $\sum t_5 =216$, $\sum t_6 =189$
- 2- Mean of $t_1=50$ 3- SSE =108
- Compare between treatments using :
- a-LSD_{0.01} if tab $t_{0.01} = 3.05$
- b- Duncan's test if SSR values = 4.32 ,4.50 ,4.62 ,4.71 and 4.77
- c-Donnet's test suppose t1 =control treatment if tab.Dt_{0.01} =3.42

