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Original Article

## Analysis of genotype-environment interaction in fennel using Sudoku design

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## Abstract

Two trials were carried out over two locations, Ramadi and Fallujah included nine genotypes of Fennel, in order to assess the interaction between genotypes (G) and locations (E) using real data from the two experiments. The nine genotypes of fennel were randomly distributed under with Sudoku square design using type-I and model-I for combined analysis. The methodologies of this type and model solution were explained in details in the current study. Genotypes were significantly differed (P<0.01) in the first location. In the combined analysis, the genotype by location (G X E) interaction was also significant (P<0.01) which is due to the effect of the genotype. It can be concluded that Sudoku square design is suitable for selecting better genotypes as a honeycomb design and to test the multi-environment trial yield. Therefore, augmented studies should be conducted to extract the efficiency of this design using other types and models embedded in this design.

Keywords: GxE interaction, Fennel, Locations, Sudoku design

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## Introduction

Soil microorganisms services have significant Sudoku is a popular combinatorial puzzle and it is the short form of the Japanese sentence "Suji Wa Dokushin Ni Kagiru" which means that the number should appear only once (Danbaba and Shehu, 2016). Sarkar and Sinha (2015) cited that Wayne Gould from Hong Kong discovered this puzzle while visiting Japan in 1997 and commenced to program computer software for this purpose, then he shared it with TIMES in London (UK) where it was first published in 2004 and ever since it was well known therefore; it consequently, had appeared in newspapers and magazine in Australia, Canada, Eastern Europe, India, and USA. Subramani and Ponnuswamy (2009) proposed the construction of Sudoku designs of order  $k = m^2$ . For applying Sudoku design in agricultural trials, they speculated linear models as a tool to analyze the data resulted from their design. Recently, Sudoku design may have been revealed as partial or NP-complete (nondeterministic polynomial time) (Béjar et al. 2012; Mahdian and Mahmoodian 2015). Likely, Danbaba and Dauran (2016) proposed a linear model and procedure for analyzing data of partially balanced Sudoku designs. In addition, Donovan et al. (2017) and Kumar et al. (2015) investigated the Sudoku extracted space-filling designs. Furthermore,

Thus, Sudoku design could be used as experimental design as special Latin square design with additional variable (internal block) without maximizing the experimental units (Sarkar and Sinha, 2015) which is represented as orthogonal Graeco-Latin square design to be orthogonal Sudoku square design (Subramani, 2013; Subramani, 2012). Li et al. (2016) proposed a facilitative and effective construction method of uniform designs based on Sudoku applied by a mixed level of factors. Recently, Subramani (2018) explained the rectangles for the construction and analysis of the Sudoku square designs. Experiments could be repeatedly laid out at years, locations (environments) or season with treatments as genotypes then data of interaction of treatments and locations, years or season extracted from these experiments could be analyzed using combined analysis. In response with this particularity, Danbaba and Shehu (2016) and Danbaba (2016) stated combined analysis to analyze data resulted from experiments conducted using Sudoku square design. Consequently, Shehu and Danbaba (2018) applied the analysis of variance method to derive the variance components for the four Sudoku square designs models. For covariance, least square method was applied to derive the sum of the square of the different effects and covariance of Sudoku square design (Shehu and Danbaba, 2018). From the other hand, a multivariate extension of various variables laid out at Sudoku designs could be extracted and done (Shehu and Danbaba, 2018). As Sudoku can be used in many situations in life, the current study was carried out by using Sudoku design in order to evaluate the performance of nine Fennel genotypes over to environments.

## **Material and Methods**

Tow field experiments were conducted at two locations viz; Ramadi and Fallujah west of Baghdad, Iraq. Sudoku square design type I was used to randomly distribute nine fennel genotypes (Figure 1).



Figure-1: Sudoku puzzle (left) and solved puzzle (right) indicated how the nine (1-9) fennel genotype randomly distributed in the two locations of the study.

Nine genotypes of the fennel were used in the current study (Table 1).

Symbol	Genotype	Origin	Introduction	Information
G1	Romanesco	Italian	Introduced by the first author	Bulbossum, long stem hollow.
G2	Amigo	Italian	from Poland in 2010	The long diameter of the main umbel
G3	Sahaa	immerced	By Elsahookie from Azuricum. Name derived	Dulhassum anni lana Tha lan
05	Sahoo	improved	from Elsahookie	Bulbossum, semi-long. The low diameter of main umbel
G4	Azuricum	Germany	By Elsahookie in 2013	
G5	Sajjet	Iranian	From the local market	long stem hollow Low diameter of the main umbel
G6	Dollap	local	Cultivated in Dollap town 135Km west Baghdad	Short stem. The low diameter of the main umbel
G7	Hannan	Jordan	By Researcher Hannan from Dept. of Horticulture named on his memory	long stem hollow Low diameter of the main umbel
G8	Shuayshae	improved	By irradiation	Short stem. The low diameter of the main umbel
G9	Di Firenze	Italian	Introduced by the first author from Poland in 2010	Bulbossum, long stem hollow. The long diameter of the main umbel

Table-1: Genetic background and some morphological characteristics the fennel genotypes used in the study.



Colu	mn	(Ramadi-L1)						(Fallujah-L2)											
bo	X		CB1			CB2			CB3			CB1			CB2		CB3		
Row box	col row	C1	C2	C3	C4	C5	C6	C7	C8	C9	C1	C2	C3	C4	C5	C6	C7	C8	C9
	R1	43	42	21	17	20	43	34	47	23	22	23	17	39	47	34	45	17	44
RB1	R2	19	16	20	43	47	30	43	39	24	48	40	16	42	43	16	25	35	25
	R3	41	32	46	35	22	24	42	15	16	35	45	44	24	23	15	16	48	40
	R4	47	14	45	33	23	18	24	43	36	45	35	25	15	24	47	44	38	17
RB2	R5	15	31	27	24	44	24	21	43	47	17	48	25	21	43	36	35	45	15
	R6	21	23	45	44	35	44	15	15	35	15	44	38	35	15	43	25	25	48
	R7	12	48	40	22	40	43	16	21	42	26	18	48	42	35	43	40	16	26
RB3	R8	41	28	16	25	43	38	47	35	16	44	16	34	16	39	21	48	26	43
	R9	43	42	23	47	20	20	39	22	43	40	26	43	47	16	22	18	44	34

Table-2. Data of secondary umbel of nine fennel genotypes grown in Ramadi (L1) and Fallujah (L2) locations.

Experiments were established during 2016-2017 season using the aforementioned design. Data were collected from plants for many agronomical traits, however, data for number secondary umbels were used as a model to explain this design and the methodology of analysis. Accordingly, data from two locations were recorded based on the solution of the Sudoku puzzle (Table 2).

Thus, data were analyzed as following steps (Locations 1):

1- Calculating the correction factor of the mean

$$c.f. = \frac{\Sigma y^2 \cdots}{n^4}$$

 $=\frac{(2552)^2}{(3)^4}=80403.75$ 

2- Calculating the total sum of square (SST)

$$SST = \sum_{i=1}^{n^2} \sum_{j=1}^{n^2} Y^2 ij - c.f. =;$$
  
= (43)<sup>2</sup>+ (42)<sup>2</sup>+.....+ (34)<sup>2</sup> -80403.75=;  
90896 -80403.75= 10492.25

3- Calculating the row box sum of squares (SSRB) (Table 3)

$$SSRB = \sum_{i=1}^{n} \frac{RB^2 i..}{n^3} - c.f. =;$$

#### Table-3: Sum of row box data- Location 1

Row box	ΣRBi
RB1	844
RB2	836
RB3	872

Thus, the analysis for location one will be  $(844)^2 + (836)^2 + (872)^2$ 

$$\frac{(044)^{-1}(050)^{-1}(072)}{3^3} - 80403.75 = 26.47$$

4- Calculating the column box sum of squares (SSCB) (Table 4)

$$SSCB = \sum_{j=1}^{n} \frac{CB^2 j_{..}}{n^3} - c_{.} f_{.} =;$$

Table-4: Sum of row box data- Location 1

Column box	CB1	CB2	CB3
ΣCBj	841	868	843

Consequently,

 $SSCB = \frac{(841)^2 + (868)^2 + (843)^2}{(3)^2} - 80403.75 = 16.77$ 

5- Calculating the row sum of squares (SSR); (Table 5)

$$SSR = \sum_{l=1}^{n^2} \frac{R^2 l}{n^2} - c.f. =;$$

#### Table-5: Sum of row data – Location 1&2

Rama	di- L1	Falluj	ah-L2
Row	$\Sigma R_{l}$	Row	$\Sigma R_{l}$
R1	290	R1	288
R2	281	R2	290
R3	273	R3	290
R4	283	R4	290
R5	276	R5	285
R6	277	R6	288
R7	284	R7	294
R8	289	R8	287
R9	299	R9	290

=

Thus, the analysis will be;

 $SSR = \frac{(290)^2 + (281)^2 + \dots + (299)^2}{(3)^2} - 80403.75 = 58.69$ 

a. extract the column sum of squares 7- Extract the genotypes sum of squares (SSG) (Table 8) (SSC) (Table 6)

$$SSC = \sum_{p=1}^{n^2} \frac{C^2 p}{n^2} - c.f. =$$

#### Table-6: Sum of column data – Location 1&2

Location		Ramadi - L1											
Column	C1	C2	C3	C4	C5	C6	C7	C8	C9				
ΣСр	282	276	283	290	294	284	281	280	282				
Location		Fallujah - L2											
ΣСр	292	295	290	281	285	277	296	294	292				
Concoque	ontly.												

Consequently,

$$SSC = \frac{(282)^2 + (276)^2 + \dots + (282)^2}{(3)^2} - 80403.75 = 25.80$$

6- Extract the sum square of sub-square or box of each row box under each column box; (Table 7)

$$SSS = \sum_{q=1}^{n^2} \frac{S^2 q_{..}}{n^2} - c_{.} f_{.} =;$$

Table-7: Sum of sub-squares data or boxes –Location 1

Location	Ra	madi ·	-L1	Fa	L2	
Sub-square	CB1	CB2	CB3	CB1	CB2	CB3
RB1	280	281	283	290	283	295
RB2	268	289	279	292	279	292
RB3	293	298	281	295	281	295

Thus,

 $SSS = \frac{(280)^2 + (281)^2 + \dots + (281)^2}{(3)^2} - 80403.75 = 68.47$ Extract the genotypes sum of squares (SSG) (Table 8

$$SSG = \sum_{k=1}^{n^2} \frac{G^2 k_{..}}{n^2} - c_{.} f_{.} =;$$

Table-8: Sum of nine genotypes data over two
locations

Genotypes	Ramadi- L1	Fallujah- L2	SUM
G1	386	344	730
G2	386	305	691
G3	309	290	599
G4	336	303	639
G5	149	290	439
<b>G6</b>	208	305	513
G7	156	346	502
<b>G8</b>	202	196	398
<b>G9</b>	420	223	643
SUM	2552	2602	5152

Accordingly,

$$SSG = \frac{(386)^2 + (386)^2 + \dots + (420)^2}{(3)^2} - 80403.75 = 9971.14$$

8- Calculating the error sum of squares (SSE)  $SSE = \sum_{i=1}^{n^2} \sum_{j=1}^{n^2} Y^2 ij - \sum_{i=1}^{n} \frac{RB^2 i}{n^3} - \sum_{j=1}^{n} \frac{CB^2 j}{n^3} - \sum_{i=1}^{n^2} \frac{R^2 l}{n^2} - \sum_{p=1}^{n^2} \frac{C^2 p}{n^2} - \sum_{q=1}^{n^2} \frac{G^2 k..}{n^2} - \sum_{q=1}^{n^2} \frac{S^2 q..}{n^2} + \frac{SY^2 ...}{n^4} = ;$ 

So, SSE will be equal to =90896 - 80430.22 - 80430.25 - 80462.44 - 80429.56 -90374.89 - 80472.22 + 402018.80=324.91

The same aforementioned steps were applied to get the same statistical information for location 2 (Fallujah); therefore ANOVA table constructed for both locations as below (Table 9);

Table-9: ANOVA Table of Sudoku design of type-1 of the mean square for both locations (Ramadi-L1 and
Fallujah-L2)

Source Of Variance	DF	DF SS		MS		F cal. (Observed)			
Source of Variance	Dr	L1	L2	L1	L2	L1	L2	L1	L2
genotypes	<b>n</b> <sup>2</sup> -1	8	8	9971.14	2221	1246.39	277.62	168.79**	1.43
Row blocks	n-1	2	2	26.47	1.21	13.24	0.61	1.79	0.003
Column blocks	n-1	2	2	16.77	33.36	8.38	16.68	1.14	0.09
Rows	n <sup>2</sup> -1	8	8	58.69	5.65	7.34	0.71	0.99	0.004
Columns	n <sup>2</sup> -1	8	8	25.80	39.21	3.23	4.90	0.44	0.03
Sub-squares (boxes)	n <sup>2</sup> -1	8	8	68.47	36.32	8.56	4.54	1.16	0.02
Error	$(n-1)[(n+1)(n^2-3)-2]$	44	44	324.91	8546.03	7.38	194.23		
Total	n <sup>4</sup> -1	80	80						



Data presented in ANOVA table indicated that genotypes possessed highly significant effect in location 1which had F calculated of 168.79 and mean squares of 1246.39 secondary umbels per the main umbel. Error mean square was extracted the low value of 7.38. The other components of variance were not significant whereas, at Location of Fallujah (L2) all components of variance were not significant. Thus, error mean square was very high whose value was 194.23 secondary umbels per main umbel (table 9). As least significant difference (LSD<sub>0.05</sub>) can be used only if F test is significant, therefore It was calculated for Location of Ramadi (L1) as it was significant for the treatments (genotypes) as follow:

## $L.S.D0.05 = t\alpha df e \sqrt{2mse/9},$

=  $2.035\sqrt{2 * 7.38/9}$ , = 2.61 for genotypes grown over location1, figure 2 showed the significant differences among genotypes at the probability of 0.05.



Figure-2. Number of secondary umbel per main umbel for the nine fennel genotypes in Ramadi location (L1) which indicated that G9 was superior overall genotypes under study (P<0.05)

#### **Combined analysis of Sudoku**

Data were recorded from two locations for nine genotypes of fennel conducted in Sudoku square design as mentioned above. Thus, the linear model was shifted to be involved in the multi-location experiment as in equation;

$$yijlmx = \mu + \theta x + \alpha i + \beta j x + \delta l x + \gamma m x + (\alpha \theta)ix + \varepsilon(ij)lmx$$

$$\begin{cases} i = 1, 2, \dots, k, number of genotypes \\ j = 1, 2, \dots, k, number of sub - squares \\ l = 1, 2, \dots, k, number of rows \\ m = 1, 2, \dots, k, number of columns \\ x = 1, 2, \dots, e, number of locations \end{cases}$$

The components of the model interpreted as below;  $y_{ijlmx}$  represents the value which observed in each plot for *l*th row and *m*th column, this value is subjected to the *i*th genotypes, *j*th sub-square (box) of the *x*th experimental location;  $\mu$  is the general mean, the symbols of  $\alpha i$ ,  $\beta j x$ ,  $\delta l x$ ,  $\gamma m x$ ,  $\theta x$  and  $(\alpha \theta) i x$ represent the main effects that influenced by *i*th genotype, *j*th sub-square, *m*th column, x location and genotype by location interaction, respectively. Residual or random error is signed by  $\varepsilon_{ijm}$ .

Thus, to complete combined analysis, the sum square of experimental location would be SSEL, SSG is the total genotype sum of squares for both locations, SSR is sum of squares of total rows for both locations, SSC is sum of squares of total columns for both locations, SSS is sum of squares of total sub-squares (boxes or sub-blocks) for both locations, SSI is sum squares of genotypes by locations and SSL is the total sum of squares of both location. To calculate these statistics indices, the following equations were applied;

$$T1 = \frac{y^2 \dots}{ek^2}, T2 = \frac{\sum_x y^2 \dots}{k^2}, T3 = \frac{\sum_l \sum_x y^2 \dots l.x}{k}, T4$$
$$= \frac{\sum_m \sum_x y^2 \dots mx}{k}, T5 = \frac{\sum_i y^2 i \dots}{ek},$$
$$T6 = \frac{\sum_x \sum_l y^2 i \dots x}{k}, T7 = \frac{\sum_j \sum_x y^2 . j.x}{k}, T8 = \sum_i \sum_j \sum_e \sum_m y^2 i j lmx$$

(Danbaba, 2016, Danbaba and Shehu, 2016)

Therefore; T1 =  $\frac{5154^2}{162}$  = 163973.6, From table 8, T2 was calculated:  $T2 = \frac{(2552)^2 + (2602)^2}{(212)^2} = 163989;$  $(9)^2$ From table 5 T3 was calculated: T3  $\frac{(290)^2 + (281)^2 + \dots + (290)^2}{2} = 164053.3$ Thus, SSR = T3 - T2; = 164053.3- 163989= 64.35 From table 6 T4 calculated:T4 was =  $\frac{(282)^2 + (276)^2 + \dots + (292)^2}{9} = 164054$ 9 Thus, SSC = T4 - T2; =164054-163989=65.01

From table8 T5 calculated:T5 was =  $\frac{(730)^2 + (691)^2 + \dots + (643)^2}{(730)^2 + (691)^2 + \dots + (643)^2} = 169847.2$ 2*x*9 Thus SSG = T5 - T2; =169847.2-163989= 5858.24 From the same table T6 was calculated: T6 = $(386)^2 + (344)^2 + \dots + (223)^2$ = 176181.19 T7 T7 was calculated: From table7, =  $\frac{(280)^2 + (281)^2 + \dots + (295)^2}{(280)^2 + (280)^2 + \dots + (295)^2} = 164093.8$ 9 Thus, SSS=T7 - T2; =164093.8-163989= 104.79 SSL = T2 - T1;163989-163973.6= 15.4321 From table2, was calculated: T8 = (43)2+

(43)2+ (42)2+.....+(44)2+(34)2=185364 Thus, SST=T8 - T1; 185364-163973.6= 21390.44, and SSI=T6 - T2 - T5 + T1; =176181.1-163989-169847.2+163973.6= 6318.45 Thus, SSE could be found by subtraction as below; SSE=T8 - T1 - T3 - T4 - T6 - T7 + 4(T2); =185364-163973.2-164053.3-164054-176181.1-164093.8+4(163989) = 8964.17

 Table-10: ANOVA table revealed combined analysis

 of Sudoku square design for nine fennel genotypes

a	10	Sum of	Mean	Fcal.
Source	df	squares	Squares	(observed)
location	e - 1 = 2 - 1 = 1	15.43	15.43	0.16
Genotypes	k-1=9-1=8	5858.24	732.28	7.84*
Rows	e(k-1) = 2(9-1)=16	64.35	4.02	0.04
Columns	e(k-1) = 2(9-1)=16	65.01	4.06	0.04
Sub- squares (boxes)	e(k-1) = 2(9-1) = 16	104.79	6.55	0.07
Genotype X location	(e-1)(k-1) = $(2-1)(9-1)$	6318.46	1120.58	12.00**
Error	e(k-1)(k-3) = 2(9-1)(9-3) = 96	8964.60	93.38	
Total	$ek^2 - 1 = 2(9)^2 - 1$ = 161	21390.44		

## Results

## Analysis of variance (ANOVA)

The Analysis of variance (table 9) and Table 10 illustrated that there is a significant difference among fennel genotypes that grown over location1 (Ramadi).

Thus, G9 was superior which showed the highest average of umbellate number per the main umbel of 46.67, followed by G1 and G2 of 42.89 and 42.89 umbellate umbel<sup>-1</sup> respectively, for each one. Whereas, G5 has the lowest number of umbellate per umbel of 16.56 umbellates umbel<sup>-1</sup>. While the genotypes that sown over location 2 did not show significant differences in the number of secondary umbel per main umbel (Table 11).

Table-11: Means	of nine fennel	genotypes	grown in
location 1&2			

Genotypes	Ramadi- L1	Fallujah- L2
G1	42.89	38.22
G2	42.89	33.89
G3	34.33	32.22
<b>G4</b>	37.33	33.67
G5	16.56	32.22
<b>G6</b>	23.11	33.89
G7	17.33	38.44
<b>G8</b>	22.44	21.78
G9	46.67	24.78
L.S.D 0.05	2.61	N.S.

 Table-12: Means of umbellate per umbel of nine

 fennel genotypes over two locations

Genotypes	Ramadi- L1	Fallujah- L2	Means
G1	42.89	38.22	40.56
G2	42.89	33.89	38.39
G3	34.33	32.22	33.28
G4	37.33	33.67	35.50
G5	16.56	32.22	24.39
G6	23.11	33.89	28.50
G7	17.33	38.44	27.89
G8	22.44	21.78	22.11
G9	46.67	24.78	35.73
L.S.D 0.05	8.92		6.31
Means	31.51	32.12	
L.S.D0.05	N.S.		

## **Combined analysis of Sudoku**

Analysis of covariance (Table 10 and 12) indicated that the genotypes of fennel significantly differed. Consequently, G1 showed the highest performance of umbellate per umbel of 40.56 umbellates umbel<sup>-1</sup>, followed by G2 of 38.39 umbellates umbel<sup>-1</sup>. Whereas, G8 has the lowest performance which gave the lowest number of umbellate of 22.11 umbellates umbel<sup>-1</sup>. Effect of location did not achieve a significant difference. Moreover, the interaction between



genotype X location displayed significant differences. Where, G9\*L1 gave the highest number of umbellate of 46.67 umbellates umbel<sup>-1</sup> followed by G1\*L1 and G2\*L1 of 42.89 umbellate umbel<sup>-1</sup> for each one. While G5\*L1 had the lowest number of umbellate of 16.56 umbellates umbel<sup>-1</sup>. Furthermore, G7\*L2 achieved the highest number of umbellate per umbel of 38.44 umbellates umbel<sup>-1</sup>, followed by G1\*L2 of 38.22 umbellate umbel<sup>-1</sup>. While G8\*L2 gave the lowest number of umbellate per umbel of 21.78 umbellates umbel<sup>-1</sup>.

## Discussion

Not many agricultural researchers have the time or ability to master the details of the complex mathematical operations, yet they all are looking forward to gain a general understanding of the logic of the designs they used. The understanding of the basics and principles of statistics will help to properly design an experiment and get logic conclusions from gained results. The current research was achieved according to real data were recorded on secondary umbel number of nine fennel genotypes that were sown in two locations in the Iraqi environment. Sudoku square design type I was used in order to detect the variation among those genotypes over the two locations. Analysis of variance of nine fennel genotypes over two locations revealed that genotypes in location1 were significantly varied at the probability of 5% (a=0.05) when used univariate Sudoku model proposed by Subramani and Ponnuswamy (2009). ANOVA technique was efficiently enhanced by Shehu and Danbaba (2018). The analysis of variance was effective to extract the effect significance variations among genotypes which supported by Subramani (2018) who used Sudoku square designs with rectangles to be applied in many fields of science. The properties of Sudoku designs made those designs were simple and efficient when used in a factorial experiment (Li et al., 2016). The combined analysis of genotypes X location (G\*L) was efficient in revealing the significant differences of some variation components nevertheless location variation effect was not significant. However, genotypes (G) and genotype X location (G\*L) were significant may due to the properties of analysis of covariance. Shehu and Danbaba (2018) discussed the analysis of variance for Sudoku models and significance of treatment who indicated that the use of this combined analysis is a

good tool to detect the variation over multi locations especially when they share common treatments.

## Conclusion

Sudoku square design Model Type I assumed as Latin square design in terms of rows, columns, and treatments (genotypes). In the current study, the aforementioned experimental design was used to derivate the variance components over multienvironments. Based on results of the current study, although G9 showed the highest number of secondary umbels per the main umbel in Ramadi (L1) only, G1 showed great stability over the two locations used in this study according to Sudoku Square design type I. yet, support studies should be applied over more divergent environments in order to detect the variation among used treatments.

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## **Contribution of Authors**

Al-Mehemdi AF: Designed the study and put the hypothesis, performed the measurement and statistical analysis Elsahookie MM: Designed the study and put the hypothesis Al-Issawi MH: Performed the measurement and statistical analysis, manuscript writing