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SOME BLOCK DESIGN WITH NESTED ROWS AND COLUMNS FOR PLANT PROTECTION RESEARCH

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Summary

The study presents the new method of constructing block designs with nested rows and columns of the type S having C property. Presented designs are dedicated to experiments with adjacent control, i.e. experiments conducted on pairs of bound experimental units. For this type of experiments, a two–phase analysis is being proposed, in the beginning with the consideration of the control treatment, and in the second phase the analysis of the differences between the values observed on control and operational units. Theoretical considerations are illustrated with the analysis of the study of plant protection.

Key words and phrases: block design with nested rows and columns, type S design, design possessing C–property, plant protection research

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1. Introduction

Due to specific character of plant protection research, when planning an experiment, one should consider a number of uncontrolled variabilities, such as duration of measurements or the use of different measuring appliances what may affect the observed feature. The fundamental rule of planning an experiment says, that the type of the experimental design should be matching quality and variability of the experimental material, technical possibilities of carrying out the procedures related to the studied phenomena and ability to carry out the observation. To protect oneself from negative influence of uncontrolled variabilities on the experiment results, it is advantageous to plan experiments of several block structures.

Within the scope of research on alternative methods of controlling the slug *Arion lusitanicus* Mabille (Arionidae) an experiment concerning the use of the biological preparation Nemaslug (Becker Underwood, Great Britain) in reducing damaging of Chinese cabbage by those pests, was planned.

Slug *A. lusitanicus* is a pest of growing economic significance (Kozłowski, 2008). It origins from the Iberian Peninsula, from where it spread as an invasion species among many European countries, including Poland. Its most often place of appearance are the areas of growing garden and farm plants. The slug damages all parts of a plant, however its favourite place of prey is germinating seed, plants in seedling phase and juvenile leaves.

Biological preparation Nemaslug contains parasitic nematode *Phasmarhabditis hermaphrodita* and is intended to control different species of slugs. This pesticide, completely safe for humans, animals and environment is available mostly in countries of Central and Northern Europe. It is being used mostly in ecological farms. In some field research conducted in England concerning the use of *P. hermaphrodita* it was possible to reduce the damage of plants such as winter wheat and Chinese cabbage, caused by slugs (Wilson et al. 1993, 1996; Hass et al. 1999; Speiser et al. 2001).

Due to lack of information on efficiency of the use of nematodes *P. hermaphrodita* in controlling Polish populations of slugs, the use of that biological pesticide is the subject of the planned research. The examined Nemaslug preparation, containing nematodes in the vigorously infective stage, easily dissolves in water, forming a suspension. It can be applied by spraying over plants, watering them or immersing the seedlings' roots in the suspension. After application *P. hermaphrodita* penetrates into the soil searching for slugs to attack on. They penetrate into the host after piercing into its back opening where they release bacteria *Moraxella osloensis* which toxins are lethal for the slug organism. After few days the slug is sick what affects its prey activity. In the planned experiment, the following three sources of variability should be considered: time of performing series of observations, ability to perform camera observation and the distance to the camera. It results from the planned use of bound experimental units (simultaneous observation of treatment unit and control treatment).

The aim of the study is to present the proposed new construction of block design with nested rows and columns, adequate to raised research issue, present the property of estimation of treatment contrasts and testing hypotheses in that design. Also showing on exemplary data that proposed scheme of distribution of treatments (experimental combinations of two factors and single control) on pairs of bound experimental units, allows for differences of the observed value on treatment unit and control unit to simplify the analysis to the analysis in block design.

2. NRC designs

In a block design with nested rows and columns (NRC design), v treatments are distributed in b_3 blocks grouped in b_1 rows and b_2 columns. Due to its structure, NRC design allows to eliminate three directions of heterogeneity originating from the experimental material. Inter alia after Kozłowska (2001), Łacka and Kozłowska (2009), Łacka *et al.* (2009a, 2009b), Kozłowska *et al.* (2010) we assume that n–dimensional vector of observation, where $n=b_1b_2b_3$ has the following form

$$\mathbf{y} = \mu\mathbf{1} + \mathbf{D}'\boldsymbol{\gamma} + \mathbf{D}_1'\boldsymbol{\rho} + \mathbf{D}_2'\boldsymbol{\varphi} + \boldsymbol{\Delta}'\boldsymbol{\tau} + \boldsymbol{\varepsilon} + \mathbf{e}, \qquad (2.1)$$

where μ is a common parameter and $\boldsymbol{\gamma}$, $\boldsymbol{\rho}$, $\boldsymbol{\varphi}$ are the vectors of random effects of blocks, rows and columns, respectively, $\boldsymbol{\tau}$ is the vector of treatment effects; $\mathbf{D}'$, $\mathbf{D}_1'$, $\mathbf{D}_2'$ and $\boldsymbol{\Delta}'$ are the design matrices of blocks, rows, columns and treatments, respectively; $\boldsymbol{\varepsilon}$ and $\mathbf{e}$ are the vectors of errors connected with experimental units and technical error, respectively; $\mathbf{1}$ is a vector of ones. This is a standard linear mixed model resulting from randomization of blocks, rows and columns in which the expected value of $\mathbf{y}$ is $\mathrm{E}(\mathbf{y}) = \mu\mathbf{1} + \boldsymbol{\Delta}'\boldsymbol{\tau}$ and the dispersion matrix has the following form

$$\begin{aligned}\mathrm{Cov}(\mathbf{y}) = {} & \sigma_\gamma^2\mathbf{D}'(\mathbf{I}_{b_3} - \mathbf{11}'/b_3)\mathbf{D}' + \sigma_\rho^2\mathbf{D}_1'(\mathbf{I}_{b_3} \otimes (\mathbf{I}_{b_1} - \mathbf{11}'/b_1))\mathbf{D}_1 + \\ & + \sigma_\varphi^2\mathbf{D}_2'(\mathbf{I}_{b_3} \otimes (\mathbf{I}_{b_2} - \mathbf{11}'/b_2))\mathbf{D}_2 + \\ & + \sigma_\varepsilon^2(\mathbf{I}_{b_3} \otimes (\mathbf{I}_{b_1} - \mathbf{11}'/b_1) \otimes (\mathbf{I}_{b_2} - \mathbf{11}'/b_2)) + \sigma_e^2\mathbf{I},\end{aligned}$$

where σ_γ^2, σ_ρ^2, σ_φ^2, σ_ε^2 and σ_e^2 denote the respective variances of the model random effects. The matrix $\mathbf{I}_x$ denotes the identity matrix of order x and the symbol $\otimes$ denotes the Kronecker product (see also Mejza and Mejza, 1994).

NRC designs considered in the study, apart from characteristic for that class of designs orthogonal block structure, have also the property of general balance, hence the analysis of experiments conducted within them can be based on the so–called stratum analysis, which, in this case, will be based on four strata: between blocks (s=1), between rows (s=2), between columns (s=3) and the bottom stratum (s=4), the so called ”rows–by–columns stratum” (see Bailey and Williams 2007; Nelder, 1965). Thanks to properties of studied design in every strata we can consider the estimation of the same set of basic contrasts $\mathbf{c}_i'\boldsymbol{\tau}$, $i=1,..., v-1$, on which the analysis of the experiment is based. The measure of the efficiency of the design in s'th strata in relation to i'th contrast is the canonical efficiency factor $\lambda_{(s)i}$, $s= 1, 2, 3, 4$; $i=1, ..., v-1$ fulfilling the condition $\mathbf{C}_{(s)}\mathbf{R}^{-1}\mathbf{c}_i = \lambda_{(s)i}\,\mathbf{c}_i$, where $\mathbf{C}_{(s)}$ for $s = 1, 2, 3, 4$ is the information matrix for stratum, whereas **R** is a diagonal matrix of the diagonal elements equal to the number of replications of successive treatments. Stratum analysis based on the analysis of basic contrasts for NRC designs connected in fourth stratum (only such designs are presented in this study) was particularly described in the article Łacka *et al.* (2009a).

In research concerning plant protection, the particular role is played by control treatment. Idiosyncrasy of that issue determines using in such experiments mostly enclosed or adjacent control, because only such approach allows including the control treatment in the statistical analysis of the experiment (see EPPO Standards PP1: 2004). The question of planning of experiments with control treatment was raised in the literature repeatedly. As it was noticed by Pearce (1960), very often the main goal of an experiment is comparing new treatments exactly with the distinguished (control) treatment. On the other hand however there exist studies which aim is to state which one of the applicable methods of plant protection is the best and the control treatment is being introduced into the experiment just to demonstrate consequences of not using the protective procedure. In first of presented situations, the major stress should be put on such planning of the experiment, that the efficiency of estimation of the contrast between control and other treatments was the highest in the bottom stratum. In second situation we are interested in the efficiency for other contrasts. In both situations, the most favourable design, except the orthogonal design, is a type S design having C–property.

Type S or “supplemented balance” designs were formally defined by Pearce in 1960 for a classic block design (earlier they were described by inter alia Cochran and Cox (1957) and Cox (1958) and since that moment they occupy a high post in literature concerning planning of experiments. Worth noticing is the study of Gupta and Kageyama (1993), where the authors present extensive tables of type S designs for $4 \le v \le 24$. They also present methods of construction of described designs using known designs BIB and GD(2). The significant feature of those designs, belonging to the class of partially balanced designs, is that information matrix for the bottom stratum has two different eigenvalues calculated with respect to matrix R, i.e. all contrasts in that stratum are being estimated only with two different efficiencies; the first of them is connected only with one contrast – with contrast $\mathbf{c}'_1\boldsymbol{\tau}$ between the emphasized (control) treatment and other treatments; the other is the efficiency of estimation of contrasts $\mathbf{c}'_i\boldsymbol{\tau}$, $i = 2,..,v-1$ between other treatments.

According to Pearce's original definition, type S designs (designs having S property) are those, in which the contrast connected with the control treatment is the most, as well as the least interesting for the researcher (see Pearce, 1960). However, the approach to this issue is not consistent in literature. Later works concerning the discussed designs focused on the first of the mentioned situations. This was the case in Gupta's and Kageyama's works (1991, 1993), after which we give the definition of the discussed design. We say that a block design with nested rows and columns has the S property, if:

$$\begin{aligned} b_1\xi_{(w)i1} + b_2\xi_{(l)i1} - \xi_{(j)i1} &= s_0 \quad \text{for} \quad i = 2,...,v \\ b_1\xi_{(w)ii'} + b_2\xi_{(l)ii'} - \xi_{(j)ii'} &= s_1 \quad \text{for} \quad i \neq i' \quad i,i' = 2,...,v \end{aligned} \tag{2.2}$$

where $\xi_{(w)ii'}$, $\xi_{(l)ii'}$, $\xi_{(j)ii'}$ mean the number of meetings of i–th and i'–th treatment respectively in rows, columns and blocks ($i \neq i' = 1,2,...,v$). While s_1 and s_0 are integers such that $s_0 \neq 0$ and $s_0 + (v-1) \neq 0$. Constructions of SNRC designs known from literature describe situations, in which all the treatments beyond control have the same number of replications. In such a case, parameters of type S NRC design (SNRC) are usually described by $\mathrm{D}(v, b_3, b_1, b_2, r_0, r, s_0, s_1)$, where r_0 and r mean the number of replications for the control treatment ($i=1$) and for the rest of the treatments ($i = 2,...,v$) respectively. The theory of SNRC designs has been expanded by Łacka and Kozłowska (2009).

The C property is strictly connected with estimation in the fourth stratum. For C designs every basic contrast is estimated in the bottom stratum with the efficiency $\lambda_{(4)i} = 1$ or $\lambda_{(4)i} = 1-\mu$, thus discretionary designs are estimated with the efficiency not lower than $1-\mu$ (Pearce *et al.* 1974). μ is described as a loss coefficient when estimating some basic contrasts.

It should be remembered, that an SNRC design will be a C design only when

$$rr_0 = s_0 b_3. \tag{2.3}$$

So far in literature only two classes of SNRC designs meeting this requirement have been known, described in the works of Gupta and Kageyama (1991), as well as Łacka and Kozłowska (2009) works. Both classes of designs have full efficiency of estimation of precisely one contrast in the fourth stratum (it is contrast connected with the control treatment), whereas the rest of the contrasts in the bottom stratum of each design are estimated with the same efficiency ($1-\mu \leq 1$, where $\mu = (v-1)\frac{r^2 - s_1 b_3}{nr}$).

We will now suggest a new construction of such designs, dedicated above all to experiments with adjacent control. It has some limitations when it comes to the number of treatments, namely, it can be used only if $v = 2l+1$, so when the number of all treatments (beyond control) is even.

Theorem 1. For any integer $l \geq 1$ there exists a SNRC design being a C design, of the parameters:

$$v = 2l+1, b_3 = \frac{l\Gamma(2l)}{\Gamma^2(l+1)}, b_1 = 2, b_2 = 2l, r_0 = \frac{\Gamma(2l+1)}{\Gamma^2(l)l}, r = \frac{\Gamma(2l)}{\Gamma^2(l)l},$$
$$s_1 = -\frac{2}{(l-1)l}\frac{\Gamma(2l-2)}{\Gamma^2(l-1)}, \quad s_0 = 2\frac{\Gamma(2l)}{\Gamma^2(l)}, \tag{2.4}$$

where $\Gamma(\cdot)$ is the value of Gamma function for $\cdot$.

For a given l, the construction of the design is connected with such distribution of treatments on experimental units, that the number of meetings of treatments in rows, columns and blocks will equal respectively

$$\xi_{(w)i1} = \frac{\Gamma(2l)}{\Gamma^2(l)}, \qquad \xi_{(l)i1} = \frac{1}{2}\frac{\Gamma(2l+1)}{\Gamma^2(l+1)}, \qquad \xi_{(j)i1} = \frac{\Gamma(2l+1)}{\Gamma^2(l)l},$$

$$\xi_{(w)ii'} = \frac{2}{l}\frac{\Gamma(2l-2)}{\Gamma^2(l-1)}, \ \xi_{(l)ii'} = 0, \ \xi_{(j)ii'} = \frac{1}{2}\frac{\Gamma(2l+1)}{\Gamma^2(l+1)} \text{ for } i=2,\ldots,v=2l+1,\ i \neq i'.$$

Therefore, the requirement (2.2) is met. It should also be noticed, that in the discussed design

$$r_0 r = \frac{\Gamma(2l+1)}{\Gamma^2(l)l}\frac{\Gamma(2l)}{\Gamma^2(l)l} = 2\frac{\Gamma^2(2l)}{l\Gamma^4(l)},$$

and

$$s_0 b_3 = 2\frac{\Gamma(2l)}{\Gamma^2(l)}\frac{1}{2}\frac{\Gamma(2l+1)}{\Gamma^2(l+1)} = 2\frac{\Gamma^2(2l)}{l\Gamma^4(l)},$$

therefore, the requirement (2.3) is also met, thus the discussed design has the C property.
Let's determine information matrices for strata for a design of the parameters (2.4); they are as follows:

$$\mathbf{C}_{(1)} = \mathbf{0}_v, \qquad \mathbf{C}_{(2)} = -\frac{1}{4}\frac{\Gamma(2l-1)}{\Gamma^2(l+1)}\begin{bmatrix} 0 & \mathbf{0}'_{2l} \\ \mathbf{0}_{2l} & \mathbf{1}_{2l}\mathbf{1}'_{2l} - 2l\ \mathbf{I}_{2l} \end{bmatrix},$$

$$\mathbf{C}_{(3)} = -\frac{1}{4}\frac{\Gamma(2l)}{\Gamma^2(l+1)}\begin{bmatrix} 0 & \mathbf{0}'_{2l} \\ \mathbf{0}_{2l} & \mathbf{1}_{2l}\mathbf{1}'_{2l} - 2l\mathbf{I}_{2l} \end{bmatrix}, \tag{2.5}$$

$$\mathbf{C}_{(4)} = \frac{1}{4}\frac{\Gamma(2l-1)}{\Gamma^2(l+1)}\begin{bmatrix} 4(2l-1)l^2 & -2l(2l-1)\,\mathbf{1}'_{2l} \\ -2l(2l-1)\mathbf{1}_{2l} & (2l-1)^2\mathbf{I}_{2l} + (\mathbf{1}_{2l}\mathbf{1}'_{2l} - \mathbf{I}_{2l}) \end{bmatrix}.$$

From the above information matrix it results that SNRC design from the class $\mathrm{D}(v, b_3, b_1, b_2, r_0, r, s_0, s_1)$ of the (2.4) parameters has the full efficiency of estimating contrast between the control treatment and the rest of the treatments in the fourth stratum, so $\lambda_{(4)1} = 1$ and $\lambda_{(s)1} = 0$ for s=1,2,3. Contrasts not connected with control are not estimated in the stratum between blocks, thus $\lambda_{(1)i} = 0$, for i=2,…,v–1. All estimated contrasts in the stratum between rows have the efficiency $\lambda_{(2)i} = \frac{1}{2(2l-1)} = \frac{1}{2(v-2)}$ for i=2,…,v–1. The third stratum has the efficiency of $\lambda_{(3)i} = \frac{1}{2}$ for any contrast not connected with control, and in the bottom stratum these contrasts have the efficiency $\lambda_{(4)i} = \frac{l-1}{2l-1} = \frac{1}{2}\frac{v-3}{v-2}$ for i=2,…,v–1.
The above design can be successfully used in near–factorial experiments, so in a situation where apart from control treatment there are combinations of at least two experimental factors, and the control itself cannot be regarded as a combination of levels of these factors.

3. Method and analysis

The experiment, which aimed at determining the influence of the nematodes *P. hermaphrodita* on limiting the activity of the slug *A. lusitanicus* preying on Chinese cabbage, as well as the extent of damage caused by it, was planned to be conducted in an air–conditioned cabin, in the temperature 16°C, RH 93%±3%, photoperiod 12/12 hours (day/night) according to the following method. 6 seedlings of Chinese cabbage variety Hilton were planned to be planted in each of 24 containers 1/3 filled with soil, each plant in the growth phase of 4 to 6 leaves. The biological preparation Nemaslug containing 30 million nematodes *P. hermaphrodita* was planned to be used in the form of spraying in doses divided into 3 parts, 0.5 ml (about 5 nematodes/cm²) and 1 ml (about 10 nematodes /cm²) each, or by immersing the plants halfway in its suspension with the addition of carboxymethyl cellulose (5g CMC/1000ml water), which task was to increase adherence of nematodes to plants, in single 3 ml (about 30 nematodes /cm²) and 6 ml (about 60 nematodes /cm²) doses. Plants in the control containers were planned to be sprayed with water. The planned experiment is a near–factorial experiment, in which five treatments were examined. Distribution of v=2l+1=5 (l=2) treatments is described by the following schema:

K	1	K	2
3	K	4	K

K	2	K	3
4	K	1	K

2	K	4	K
K	1	K	3

It is a schema of a block design with nested rows and columns from the class $D(5,3,2,4,1\,2,3,12,-1)$, where 1 and 2 stand for spraying lower and higher dose, respectively, 3 and 4 stand for immersing in suspension of lower and higher concentration, and K stands for adjacent control. Two slugs *A. lusitanicu*s starving for 48 hours were planned to be placed in each container. After determining masses of slugs used in the experiment, damage of the plants was planned to be observed every other day, using a five–grade scale of damages (0, 25, 50, 75 and 100% of damaged plant surface), as well as activity of preying and state of the health of the slugs.

With the above plan of distribution of treatments on experimental units, information matrices for the design strata are as follows:

$$\mathbf{C}_{(1)} = \mathbf{0}_5, \qquad \mathbf{C}_{(2)} = \frac{1}{8}\begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 3 & -1 & -1 & -1 \\ 0 & -1 & 3 & -1 & -1 \\ 0 & -1 & -1 & 3 & -1 \\ 0 & -1 & -1 & -1 & 3 \end{bmatrix},$$

$$\mathbf{C}_{(3)} = 3\mathbf{C}_{(2)}, \qquad \mathbf{C}_{(4)} = \frac{1}{8}\begin{bmatrix} 48 & -12 & -12 & -12 & -12 \\ -12 & 9 & 1 & 1 & 1 \\ -12 & 1 & 9 & 1 & 1 \\ -12 & 1 & 1 & 9 & 1 \\ -12 & 1 & 1 & 1 & 9 \end{bmatrix}.$$

Thus $\lambda_{(2)1} = \lambda_{(3)1} = \lambda_{(1)i} = 0$ for $i = 1,2,3,4$, for $i = 2,3,4$ $\lambda_{(2)i} = 1/6$, $\lambda_{(3)i} = 1/2$, $\lambda_{(4)i} = 1/3$ and $\lambda_{(4)1} = 1$.

As it can be seen, in a design of the (2.4) parameters, observations are conducted on pairs of experimental units. It is a classic design with adjacent control, which is an experimental situation in which each treatment unit is adjacent to a control unit. It is worth emphasising, that the variation inside columns being in the design from theorem 1 pairs of bound experimental units, is different from variation between bound pairs, i.e. is different inside columns than between them. Observations on pairs of plots can be used here to determine the difference or proportion of values observed on the treatment plot and adjacent control plot. In the case of such determined variable, analysis is easier than in the case of the observed variable, for which the observation model is the model of near–factorial experiment of block design with nested rows and columns. The above SNRC design guarantees full efficiency of estimation of the contrast between control treatment and the rest of the treatments in the fourth stratum. The rest of the contrasts, that is both the contrast between factor A levels, as well as contrasts between factor B levels within a given factor A level, are implicit in strata between rows and between columns, and efficiency of estimation of these contrasts in the bottom stratum may not be satisfactory for the researcher. Determining differences for pairs of bound units leads to analysis of experiment in block design of complete blocks. It is an orthogonal design for a two–factorial experiment, allowing for estimation of all contrasts with full efficiency. Such an approach significantly simplifies statistical analysis of the experiment, but also limits conclusions to determined differences. For the full assessment of research conducted, however, after performing an analysis taking into account control as a separate treatment, it is worth comparing differences for experimental combinations observed on bound units.

Stratum analysis for the discussed experiment will be based on a set of four basic contrasts and conducted for demonstration purposes on exemplary data. In research, the answer to the question whether using different doses of biological preparation Nemaslug in the form of spraying or immersing plants will be effective is interesting, hence the planned comparison between the control treatment and the rest of the treatments expressed with the contrast $\mathbf{c}_1'\boldsymbol{\tau} = \frac{\sqrt{6}}{4}[4,-1,-1,-1,-1]\boldsymbol{\tau}$. Another contrast $\mathbf{c}_2'\boldsymbol{\tau} = \frac{\sqrt{3}}{2}[0,1,1,-1,-1]\boldsymbol{\tau}$ is a comparison of two methods of applying the Nemaslug preparation, whereas the contrasts $\mathbf{c}_3'\boldsymbol{\tau} = \frac{\sqrt{6}}{2}[0,1,-1,0,0]\boldsymbol{\tau}$ and $\mathbf{c}_4'\boldsymbol{\tau} = \frac{\sqrt{6}}{2}[0,0,0,1,-1]\boldsymbol{\tau}$ are comparisons of effects of using a lower and higher dose of the examined preparation, respectively in the case of using spraying and immersing. Analysis of variance runs in the stratum between columns and in the bottom stratum. In both cases, for exemplary data from the introductory experiment performed in Institute of Plant Protection – NRI in Poznań, the general null hypothesis $H_{0(s)}$: $\mathbf{C}_{(s)}\boldsymbol{\tau}=\mathbf{0}$ for s=3,4 at the significance level of α=0.05, has been rejected. It should be noticed, that only in the fourth stratum all the interesting contrasts are estimated. A detailed analysis of contrasts in the bottom stratum on these exemplary data has been presented in table 1. With such a complicated structure of the experimental design, for two contrasts highly significant variety has been shown. The average value in the case of spraying the lower dose is 23.5%, whereas 30.8% in the case of higher dose. When immersing the plant in the suspension of lower concentration, on average 35.3% has been shown, and in the case of higher concentration– 36.5%.

Table 1. Stratum analysis in NRC design – bottom stratum (s=4)

Source	**Degrees of freedom**	**Sum of squares**	**F**	**p**
Treatments	4	4030.666667	811.5436	0.0000003
contrast 1 $\mathbf{c}_1'\boldsymbol{\tau}$	1	3927.041667	3162.718	0.0000000
contrast 2 $\mathbf{c}_2'\boldsymbol{\tau}$	1	76.5625	61.66107	0.0005378
contrast 3 $\mathbf{c}_3'\boldsymbol{\tau}$	1	26.28125	0.45302	0.5307540
contrast 4 $\mathbf{c}_4'\boldsymbol{\tau}$	1	0.78125	0.629195	0.4636120
Error	5	6.208333		
Total	9	4036.875		

Further analysis is proposed to be conducted for difference of observations on pairs of bound units. In this case, the analysis is conducted in the block design of three complete blocks and four treatments. The observations are differences between values of the attribute observed on the treatment plot and control plot. By adopting mixed observation model (see Caliński and Kageyama , 2000) it can be noticed, that statistical analysis will run only in the bottom stratum (intra block analysis). In this stratum, all the contrasts are estimated with full efficiency. As a set of basic contrasts we take: comparison between two methods of application of the Nemaslug preparation $\dot{\mathbf{c}}'_1\,\dot{\boldsymbol{\tau}} = \frac{\sqrt{3}}{2}[1,1,-1,-1]\dot{\boldsymbol{\tau}}$ and comparison of using lower and higher dose of the preparation in the case of spraying $\dot{\mathbf{c}}'_2\,\dot{\boldsymbol{\tau}} = \frac{\sqrt{6}}{2}[1,-1,0,0]\dot{\boldsymbol{\tau}}$ and in the case of immersing $\dot{\mathbf{c}}'_3\,\dot{\boldsymbol{\tau}} = \frac{\sqrt{6}}{2}[0,0,1,-1]\dot{\boldsymbol{\tau}}$. After rejecting the null hypothesis at the significance level of α=0.05, a detailed analysis of contrasts for exemplary data has been conducted. This analysis, presented in table 2, shows that for two contrasts, high significance has been shown. In this case, the average decrease of value of the observed attribute in proportion to control was respectively 33% in the case of using spraying in the lower dose, 23.33% for spraying in the higher dose, and 22% and 20.67% for immersing in the preparation of lower and higher concentration, respectively.

Table 2. Particular analysis in block design

Source	Degrees of freedom	Sum of squares	F	p
Treatments	3	282.9166667	6.830986	0.023144
contrast 1 $\dot{\mathbf{c}}'_1\,\dot{\boldsymbol{\tau}}$	1	140.0833333	10.14688	0.018946
contrast 2 $\dot{\mathbf{c}}'_2\,\dot{\boldsymbol{\tau}}$	1	140.1666667	10.15292	0.018923
contrast 3 $\dot{\mathbf{c}}'_3\,\dot{\boldsymbol{\tau}}$	1	2.6666667	0.193159	0.675688
Error	6	82.8333333		
Total	9	365.75		

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