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# Mixture Experiments and their Application in Agricultural Research

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**Abstract**

The present study was designed to show the applicability of Mixture designs in Agricultural Research System and to fit an appropriate mixture regression model making response variables as functions of the proportions of the mixture components. Data on four components namely neem oil, garlic oil, clove oil and tobacco extract (ml) were collected from field experiment conducted by Honeybee Research Institute, NARC. The main goal of the experiment was to check whether blending two components have any synergistic effect on honey yield. The results of the mixture regression showed that the positive interaction coefficients of blending components neem oil*garlic oil (1.10) and neem oil*tobacco extract (6.73) were smaller than their individual coefficients which indicated that combining these components will not have significant impact on honey yield. Negative interaction coefficients of neem oil*clove oil (-5.11) and garlic oil*clove oil (-15.86) signaled no significance meaning that they were antagonistic towards one another and will not contribute in increasing honey yield. The positive interaction coefficient of the blending component clove oil*tobacco extract (16.99) shows synergistic effect of these components on honey yield implying that honey yield can increase when clove oil and tobacco extract are blended.

**Keywords:** Mixture design, mixture regression model, components, honey yield

## Introduction and Background

In mixture experiments a product is formulated by blending proportions of different components and ingredients together For instance if we wish to obtain an optimal taste of a pancake then the components of interest might be the proportions of flour, milk, eggs, oil and baking powder in a blend. Scheffe (1958) was the first to introduce the concept of mixture experiments and their analysis. Piepel and Cornell (1994) have discussed the planning of a mixture experiment which involves defining objective of the experiment, selecting the mixture components, identifying constraints on the mixture experiments and the response variable to be measured, proposing a mathematical model for modelling the response data and choosing an appropriate experimental design to fit the proposed model.

Various examples of mixture experiments and their applications in agriculture can be

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found in literature that includes yield measurement of a crop due to applications of various mixtures of fertilizers or pesticides. Batra *et al.* (1999) have used mixture experiments in the analysis of agricultural experiments with the application of fixed amount of fertilizer to different crop growth stages. In animal husbandry feeding trials are useful to study the response on milk yield.

Mixture experiments are also pertinent to use in the field of food sciences for the evaluation of the products in terms of taste, flavor, aroma etc. Deka *et al.* (2001) applied mixture methodology for quality evaluation of mixed fruit juice/pulp ready to serve beverages.

Begon *et al.* (1990) and Vandermeer (1989) have talked about the use of crop mixtures for competition studies in plant ecology.

Lapointre *et al.* (2008) stated that mixure designs and their applications are useful for insect rearing programs where diet optimization is desired for researcher selected ceiteria.

Peace (1993) has discussed about choosing a design and experiments in the book Taguchi methods. Spitters (1983) stated that yield of grain per unit area is an essential measure of mixture performance in such experiments although it represents only a part of total plant biomass and may not fully reflect the result of competition between species in mixture

Cornell (2002) and SAS (2003) have discussed the analysis and modeling of mixture experiments. Cornell (1990), Montgomery and Voth (1994), Meyers and Montgomery (2002) and John (1984) have worked a lot on the design and analyses of mixture experiments. An experiment was conducted to study the effect of nitrogen applied in splits at different crop growth stages on yield of paddy crop at Rice Research Station, Behrampur (Orissa) in 1971.

Keeping in view the importance of mixture design and mixture experiments, a study was planned to show the applicability of mixture design in different fields of agriculture and to fit an appropriate mixture regression model making response variables as functions of the proportions of the mixture components.

## Methodology

Data on four components namely neem oil (ml), garlic oil (ml), clove oil(ml) and tobacco extract (ml) individually and in combinations such as (neem oil + garlic oil, neem oil + clove oil, neem oil+ tobacco extract, garlic+ clove, garlic+ tobacco, clove+ tobacco) with different percentage proportions 0, 0.3,0.5,0.7, 1 were collected from field experiment conducted by Honeybee Research Institute, NARC. In a mixture experiment four components namely neem oil, garlic oil, clove oil and tobacco extract were blended and applied to the bee hives in order to determine increase in honey yield. Custom mixture design approach in MINITAB was used to create a design. Table 1 shows these components when mixed together made a total of six combinations as (neem oil + garlic oil, neem oil + clove oil, neem oil + tobacco extract, garlic + clove, garlic + tobacco, clove+ tobacco) with different percentages such as (0.70, 0.30), (0.50, 0.50) and (0.30,0.70) repeated respectively for each blend. The sum for each run of the mixture is equal to one and the component values are interpreted as proportions (Cornell 2002).

Design points in runs 1 to 4 are referred to pure blends comprising only one component mixture. Runs 5 to 66 are concerned to be binary blends belonging to the mixture of two components (Table 1). Simplex design plots are useful for visualizing the mixture design space or a slice of the design space for more than three components. A matrix plot displaying four simplex design plots in a single page layout was created using graph options in MINITAB software after analyzing data from mixture experiment. Fig 1 shows a matrix of simplex design plots in proportions. Each plot shows respectively a three components triangle keeping the effect of the fourth component as constant. The vertices or the corners of the triangle, denoted by 1 represent pure blends consisting of only a particular component and the rest of the components set to '0' as in runs 1 to 4. '2' indicates a binary blend consisting of two components as in runs 5 to 66.

**Table 1: Four components (neem oil, garlic oil, clove oil, tobacco extract) custom mixture design with 0, 0.3, 0.5, 0.7, 1 proportion**

| Run | Point type | Blend Type | neem | garlic | clove | Tobacco |
|---|---|---|---|---|---|---|
| 1 | Vertex | Pure | 1 | 0 | 0 | 0 |
| 2 | Vertex | Pure | 1 | 0 | 0 | 0 |
| 3 | Vertex | Pure | 1 | 0 | 0 | 0 |
| 4 | Vertex | Pure | 0 | 1 | 0 | 0 |
| 5 | Vertex | Pure | 0 | 1 | 0 | 0 |
| 6 | Vertex | Pure | 0 | 1 | 0 | 0 |
| 7 | Vertex | Pure | 0 | 0 | 1 | 0 |
| 8 | Vertex | Pure | 0 | 0 | 1 | 0 |
| 9 | Vertex | Pure | 0 | 0 | 1 | 0 |
| 10 | Vertex | Pure | 0 | 0 | 0 | 1 |
| 11 | Vertex | Pure | 0 | 0 | 0 | 1 |
| 12 | Vertex | Pure | 0 | 0 | 0 | 1 |
| 13 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 |
| 14 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 |
| 15 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 |
| 16 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 |
| 17 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 |
| 18 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 |
| 19 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 |
| 20 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 |
| 21 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 |
| 22 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 |
| 23 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 |
| 24 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 |
| 25 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 |
| 26 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 |
| 27 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 |
| 28 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 |
| 29 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 |
| 30 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 |
| 31 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 |
| 32 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 |
| 33 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 |
| 34 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 |
| 35 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 |
| 36 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 |
| 37 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 |
| 38 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 39 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 |
| 40 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 |
| 41 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 |
| 42 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 |
| 43 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 |
| 44 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 |
| 45 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 |
| 46 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 |
| 47 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 |
| 48 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 |
| 49 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 |
| 50 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 |
| 51 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 |
| 52 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 |
| 53 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 |
| 54 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 |
| 55 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 |
| 56 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 |
| 57 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 |
| 58 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 |
| 59 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 |
| 60 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 |
| 61 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 |
| 62 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 |
| 63 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 |
| 64 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 |
| 65 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 |
| 66 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 |

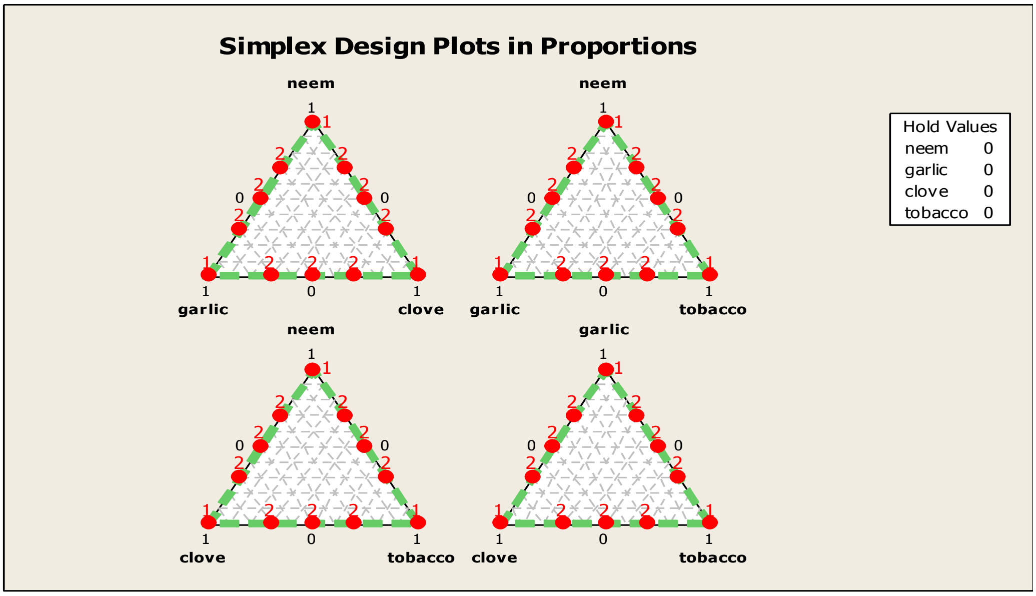


Figure 1: A matrix of simplex design plots in proportions

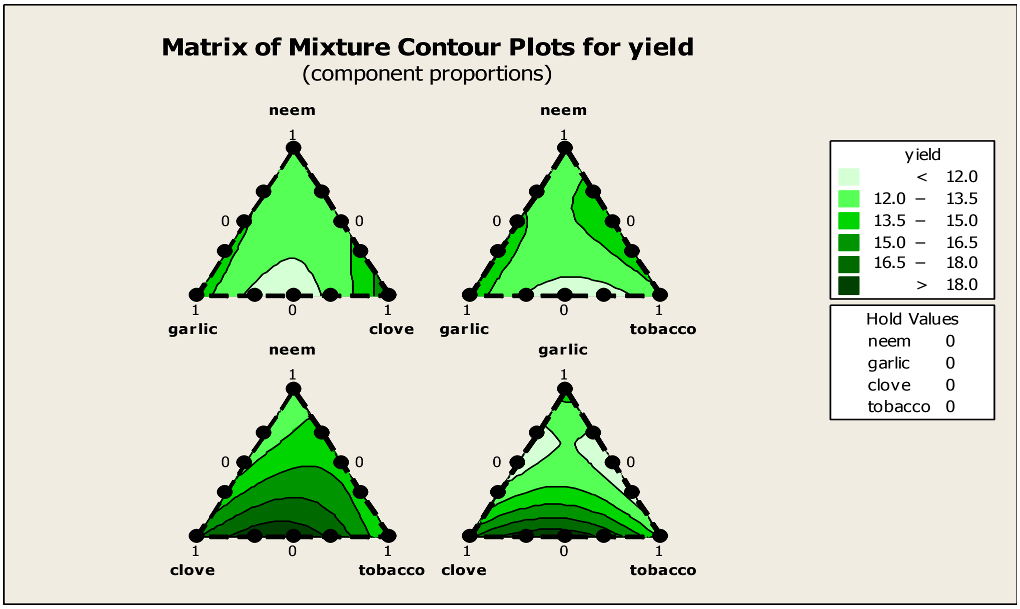


**Figure 2: Matrix of mixture contour plots for yield**

Fig 2 shows a matrix of four contour plots for honey yield versus different combinations of the four components (neem oil, garlic oil, clove oil and tobacco extract). The contour lines help define the shaded regions more sharply. In this figure, the third triangle shows darker regions indicating higher yield-values. These higher yield-values are the result of the synergistic effect of the components clove oil and tobacco extract.

A Statistical technique called Mixture Regression was used to analyze and fit multiple regressions to data collected from the experiment. Analysis and modeling of mixture experiments have been discussed by Cornell (2002) and SAS (2003). Table 2 shows response (Honey yield) data from applications of 4 components ( neem oil, garlic oil, clove oil, tobacco extract) in a mixture experiment. Design of Experiments and analyze mixture design options were selected from the Stat menu of MINITAB software. Four columns namely neem oil, garlic oil, clove oil and tobacco extract and a response column "honey yield "were selected for analysis.

**Table 2: Response (Honey yield) data from applications of 4 components (neem oil, garlic oil, clove oil, tobacco extract) in a mixture experiment**

| Run | Point type | Blend Type | neem | garlic | clove | tobacco | yield Kg |
|---|---|---|---|---|---|---|---|
| 1 | Vertex | Pure | 1 | 0 | 0 | 0 | 12 |
| 2 | Vertex | Pure | 1 | 0 | 0 | 0 | 13 |
| 3 | Vertex | Pure | 1 | 0 | 0 | 0 | 11.9 |
| 4 | Vertex | Pure | 0 | 1 | 0 | 0 | 14.5 |
| 5 | Vertex | Pure | 0 | 1 | 0 | 0 | 15 |
| 6 | Vertex | Pure | 0 | 1 | 0 | 0 | 15 |
| 7 | Vertex | Pure | 0 | 0 | 1 | 0 | 16.5 |
| 8 | Vertex | Pure | 0 | 0 | 1 | 0 | 17 |
| 9 | Vertex | Pure | 0 | 0 | 1 | 0 | 16.1 |
| 10 | Vertex | Pure | 0 | 0 | 0 | 1 | 14 |
| 11 | Vertex | Pure | 0 | 0 | 0 | 1 | 15 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | Vertex | Pure | 0 | 0 | 0 | 1 | 14.7 |
| 13 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 | 12 |
| 14 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 | 14 |
| 15 | Edge Centroid | Binary | 0.7 | 0.3 | 0 | 0 | 12 |
| 16 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 | 14.7 |
| 17 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 | 16 |
| 18 | Edge Centroid | Binary | 0.5 | 0.5 | 0 | 0 | 15 |
| 19 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 | 13 |
| 20 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 | 14 |
| 21 | Edge Centroid | Binary | 0.3 | 0.7 | 0 | 0 | 11 |
| 22 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 | 12 |
| 23 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 | 12 |
| 24 | Edge Centroid | Binary | 0.7 | 0 | 0 | 0.3 | 14.7 |
| 25 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 | 18 |
| 26 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 | 18 |
| 27 | Edge Centroid | Binary | 0.5 | 0 | 0 | 0.5 | 17.4 |
| 28 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 | 11 |
| 29 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 | 12 |
| 30 | Edge Centroid | Binary | 0.3 | 0 | 0 | 0.7 | 11 |
| 31 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 | 12 |
| 32 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 | 11 |
| 33 | Edge Centroid | Binary | 0.7 | 0 | 0.3 | 0 | 12 |
| 34 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 | 14.4 |
| 35 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 | 16 |
| 36 | Edge Centroid | Binary | 0.5 | 0 | 0.5 | 0 | 15 |
| 37 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 | 13 |
| 38 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 | 12 |
| 39 | Edge Centroid | Binary | 0.3 | 0 | 0.7 | 0 | 12 |
| 40 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 | 11 |
| 41 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 | 12 |
| 42 | Edge Centroid | Binary | 0 | 0.7 | 0.3 | 0 | 11 |
| 43 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 | 12.6 |
| 44 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 | 13 |
| 45 | Edge Centroid | Binary | 0 | 0.5 | 0.5 | 0 | 12 |
| 46 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 | 12 |
| 47 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 | 11 |
| 48 | Edge Centroid | Binary | 0 | 0.3 | 0.7 | 0 | 11 |
| 49 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 | 11 |
| 50 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 | 11 |
| 51 | Edge Centroid | Binary | 0 | 0.7 | 0 | 0.3 | 11 |
| 52 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 | 12 |
| 53 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 | 12 |
| 54 | Edge Centroid | Binary | 0 | 0.5 | 0 | 0.5 | 13 |
| 55 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 | 11 |
| 56 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 | 10.5 |
| 57 | Edge Centroid | Binary | 0 | 0.3 | 0 | 0.7 | 10.5 |
| 58 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 | 18 |
| 59 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 | 19.5 |
| 60 | Edge Centroid | Binary | 0 | 0 | 0.7 | 0.3 | 20.5 |
| 61 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 | 21 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 62 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 | 20.5 |
| 63 | Edge Centroid | Binary | 0 | 0 | 0.5 | 0.5 | 20 |
| 64 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 | 15 |
| 65 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 | 14 |
| 66 | Edge Centroid | Binary | 0 | 0 | 0.3 | 0.7 | 17 |

## Results and discussion

### Regression for Mixtures: yield (kg) versus neem oil, garlic oil, clove oil and tobacco extract

Table 3 shows estimated regression coefficients and table 4 depicts analysis of variance for honey yield (kg) in component proportions. The parameter estimate for clove oil is greater than neem oil, garlic oil and tobacco extract (table 3), it can be concluded that clove oil will be the most effective single component in increasing honey yield. The positive interaction coefficients of blending components neem oil * garlic oil (1.10) and neem oil *tobacco extract (6.73) are smaller than their individual coefficients which indicates that combining these components will not have significant impact on honey yield. Negative interaction coefficients of neem oil *clove oil (-5.11) and garlic oil *clove oil (-15.86) indicate no significance and antagonism towards one another implying that these components when blended together will not augment honey yield. The positive interaction coefficient of the blending component clove oil*tobacco extract (16.99) shows synergistic effect of these components on honey yield implying that honey yield can increase when clove oil and tobacco extract are blended.

Traditionally different statistical techniques such as descriptive statistics, analysis of variance etc have been used for the quality evaluation of honey. Iftikhar *et al.* (2011) used analysis of variance technique for the comparison and quality of different samples of honey from different areas of Pakistan. Lazarova *et al.* (2010) made use of descriptive statistics for studying botanical origin and inorganic content of bee honey in Northeast Bulgaria. However less attention has been paid on the use of mixture design for the evaluation of honey. Literature shows the applicability of mixture design and experiments in different fields of agriculture. An example from MINITAB software version 15.1 (2006) shows an application of mixture design which was used to determine how the proportions of three ingredients in an herbal blend household deodorizer affect the acceptance of the product based on scent. The three components were neroli oil, rose oil, and tangerine oil. The results of the mixture regression implied that the two blend mixture of the components neroli oil and tangerine oil was synergistic or complimentary with each other having the highest acceptance level. The importance of mixture design for the quality improvement of honey has been highlighted by Cano *et al.* (2006). An example from food science and technology states the application of mixture design for optimization of fruit punch containing (lemon, orange and mango) (Kumar *et al.,* 2010).

Regression in the analysis of variance (table 4) tests whether the terms in the model ie the four components alone and their combinations have any effect on the response variable (honey yield). The regression model is significant at $p \leq 0.01$ which means that at least one of the terms in the regression equation makes a significant impact on the response variable. Regression is broken into different orders of terms in the model, linear and quadratic. The p values for all effects are less than 0.05. There are significant linear and quadratic effects for components.

**Table 3: Estimated regression coefficients for yield (kg)(component proportions)**

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| neem oil | 12.35 | 0.9220 | * | * |
| garlic oil | 14.41 | 0.9220 | * | * |
| clove oil | 16.29 | 0.9220 | * | * |
| tobacco extract | 13.10 | 0.9220 | * | * |
| neem oil*garlic oil | 1.10 | 3.9958 | 0.28 | 0.784 |
| neem oil*clove oil | -5.11 | 3.9958 | -1.28 | 0.207 |
| neem oil*tobacco extract | 6.73 | 3.9958 | 1.68 | 0.098 |
| garlic oil*clove oil | -15.86 | 3.9958 | -3.97 | 0.000 |
| garlic oil*tobacco extract | -10.49 | 3.9958 | -2.63 | 0.011 |
| clove oil*tobacco extract | 16.99 | 3.9958 | 4.25 | 0.000 |

R-Sq = 64.97%   R- Sq(pred) = 52.93%   R-Sq(adj) = 59.34%

**Table 4: Analysis of variance for honey yield (kg) (component proportions)**

| Source | DF | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|
| Regression | 9 | 328.69 | 36.5216 | 11.54 | 0.000 |
| Linear | 3 | 35.69 | 11.8951 | 3.76 | 0.016 |
| Quadratic | 6 | 229.52 | 38.2529 | 12.09 | 0.000 |
| Residual Error | 56 | 177.19 | 3.1641 | | |
| Lack-of-Fit | 12 | 147.68 | 12.3066 | 18.35 | 0.000 |
| Pure Error | 44 | 29.51 | 0.6708 | | |
| Total | 65 | | | | |

## Conclusion and recommendation

Mixture regression technique has proven to be functional in finding the synergistic effect of the components clove oil and tobacco extract on honey yield. This implies that honey yield can rise by blending these two components.

Mixture experiments and their analysis are applicable to a wide range of agricultural field experiments such as split application of fertilizers, intercropping experiments where the interest of the experimenter is to find the best crop mixture, feeding trials in animal nutritional experiments etc.

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