Distribution fitting 13. Analysis of independent, multiplicative effect of factors. Application to effect of essential oils extracts from plant species on bacterial species. Application to the factors of antibacterial activity of plant species

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Abstract. A factor effect study was conducted on a set of observations at the contingency of a series of plant species and bacteria species regarding the antibacterial activity of essential oil extracts. The study reveals a very good agreement between the observations and the hypothesis of independent and multiplicative effect of plant and bacteria species factors on the antibacterial activity. Shaping of the observable to a Negative Binomial distribution allowed the separation of two convoluted Gamma distributions in the observable further assigned to the distribution of factors. Statistics of the Gamma distribution allowed estimating the ratio between diversity of plants factors and bacteria factors in the antibacterial activity of essential oils extracts.

Keywords: factor analysis; Negative Binomial distribution; Poisson distribution; Gamma distribution; differential entropy; plant species; bacteria species; antibacterial activity

Introduction

Recoding the data from an observation may provide different types of outcomes: binary, multinomial, or ordinal, if are seen different states of the observed; absolute or relative values if a measurement scale or ratio are used; more, the data may come from a discrete or continuous pool of possible values and our observation may or may not catch the true or whole domain of the observable and some times we can miss even its type. This is the main reason for which assumptions are made and statistics are involved to check the assumptions at a certain level of confidence.

Going forward with the observation, experiments are designed in order to collect the data in certain imposed conditions allowing us to extract the information regarding the observed variable or phenomena.

Agreement between a model and a series of observations usually implies estimation of unknown (unforeseen) parameters about which we may assume that are characteristics of the population of whole possible observations from which the sample of observations were drawn (Jäntschi, 20091). Measuring the agreement between the model and the series of observation is a matter of statistics, requires a given specific model and a series of statistical tests, designed for general or specific cases give different measures of the agreement, based too on other certain assumptions regarding the observed phenomena (Jäntschi & Bolboacă, 20092). The most common assumption is the assumption of normality and it comes from the common sense that most of the data that we observe are normal distributed, or it comes from populations that are normal distributed. But even in this case a global agreement of all statistics involved occurs far less than our expectations and should apply when a conclusion regarding the agreement between the observation and the model are drawn (Bolboacă & Jäntschi, 20093).

Not always the measurement and the analysis are conducted by the same people or same group of people. In certain cases, the data may suffer alteration processes during the way from observation or experiment to analysis. Certain statistics were developed to cover this aspect.
too, and to measure the probability that data may not come from an impartial observation (Jäntschi & others, 2009a4).

Certain conditions imposed to the experiment collecting the observation may reshape the original distribution of the observed population, and by using distribution analysis is possible to obtain this new shape, specifically to the experiment in which the observation were made (Jäntschi & others, 2009h5). In other cases, subject of observation may provide unsymmetrical shape of observed values, giving weight to higher (or lower) values disfavouring the opposite case and this fact can be revealed too (Marta & others, 20099).

Contingency of factors in the observable is one of the most important aspects of experimental studies, and may indicate or proof the way in which a process should be conducted in order to maximize the outcome (Jäntschi & Bălan, 20093). Field experiments are usually conducted in environmental conditions which are not in control of the observer, and knowing the inferences coming from changing of these conditions is essential to the parsimony of the factors affecting the observable (Bălan & others, 20095).

Other aspects such as special cases in which values recorded in an ordered outcome category based experiment may provide useful knowledge about the corrections that should be made on the values associated with the categories (Stoenoiu & others, 20096; Stoenoiu & others, 201010).

Analysing the data regarding the morphology of plants spread in a certain region provide knowledge about the effect of adaptation to certain conditions of living plant species (Jäntschi & Bolboacă, 201111). Other effects of environmental conditions in which plants forced to be adapted can be found from distribution of chemical compounds in plant species (Jäntschi & others, 201112).

The present study takes into the analysis the distribution of the antibacterial activity at the contingence between plant species and essential oil extracts of plant species. The aim of the study is to reveal how the biological activity is influenced by plant and bacteria species and to infer the distribution of biological activity from plant and bacteria species.

Material

The data regarding the antibacterial activity of essential oils of plants measured as inhibition zones by using disc-diffusion method are taken from an experimental study (Soković & others, 200713) and are given in Table 1.

| Antibacterial activity - inhibition zones in mm | Plant species | Bacteria species |
|-----------------------------------------------|--------------|-----------------|
| M.s. | M.p. | C.l. | C.a. | M.c. | L.a. | O.b. | S.o. | O.v. | T.v. |
| M. flavus | 25 | 25 | 19 | 19 | 13 | 22 | 23 | 15 | 35 | 30 |
| B. subtilis | 24 | 22 | 18 | 18 | 12 | 20 | 22 | 14 | 34 | 28 |
| S. epidermidis | 20 | 20 | 14 | 14 | 12 | 18 | 18 | 12 | 30 | 26 |
| S. aureus | 22 | 20 | 16 | 14 | 10 | 18 | 18 | 12 | 32 | 28 |
| S. enteritidis | 20 | 20 | 13 | 10 | 9 | 16 | 18 | 10 | 27 | 24 |
| S. typhimurium | 18 | 17 | 11 | 8 | 8 | 16 | 16 | 10 | 25 | 20 |
| E. coli | 16 | 16 | 12 | 9 | 9 | 14 | 14 | 10 | 26 | 22 |
| E. cloacae | 14 | 14 | 9 | 9 | 9 | 12 | 12 | 10 | 25 | 22 |
| L. monocytogenes | 16 | 13 | 9 | 8 | 8 | 10 | 11 | 9 | 25 | 18 |
| P. mirabilis | 10 | 11 | 0 | 0 | 0 | 7 | 8 | 0 | 22 | 18 |
| P. aeruginosa | 10 | 10 | 0 | 0 | 0 | 6 | 8 | 0 | 20 | 16 |

M.s.: Mentha spicata; M.p.: Mentha piperita; C.l.: Citrus limon; C.a.: Citrus aurantium; M.c.: Matricaria chamomilla; L.a.: Lavandula angustifolia; O.b.: Ocimum basilicum; S.o.: Salvia officinalis; O.v.: Origanum vulgare; T.v.: Thymus vulgaris;
On the data from Table 1 an analysis of independence were conducted using Chi-square test. The analysis revealed that for P. mirabilis and P. aeruginosa bacteria species the hypothesis of independence cannot be accepted (d.f. Plants = 9; X^2_{P. mirabilis} = 29.1; X^2_{P. aeruginosa} = 26.2; p_{x^2}(P. mirabilis) < 1‰; p_{x^2}(P. aeruginosa) < 2‰) and therefore were withdrawn from further analysis.

Without these two bacteria, the analysis of independence was conducted again, when the X^2 statistic decreased dramatically (X^2(10-1 plants, 11-1 bacteria) = 69.3; X^2(10-1 plants, 9-1 bacteria) = 8.5; p_{x^2}(8.5,72) > 0.9999).

As can be observed, the data given in Table 1 are integers (millimetres) and then even if the true distribution of the observable is not a discrete one, the observed distribution is always discrete when we use an instrumentation that has a precision limit of one millimetre.

For simplicity (an in the mean time for generality), let's note with 'm' the number of rows - bacteria (m = 9) and with 'n' the number of cols - plants (n = 10). Let us recall that the expectancies under assumption of independence between rows and cols are given by (where O_{i,j} are the observed cell from \text{i}^{th} row and \text{j}^{th} column in Table 1):

\[ E_{i,j} = \frac{1}{n} \sum_{i=1}^{m} \sum_{j=1}^{n} O_{i,j} / \left( \sum_{i=1}^{m} \sum_{j=1}^{n} O_{i,j} \right) \]

**Method**

Analysis of multiplicative effect of factors under assumption of normal distributed observed absolute error (Fisher, 1923\textsuperscript{14}; Bolboacă & others, 2011\textsuperscript{15}) give the following equation relating the "a_i" - rows factors and "b_j" - cols factors:

\[ E_{i,j} = a_{i} \cdot b_{j}; S^{2} = \sum_{i=1}^{m} \sum_{j=1}^{n} (O_{i,j} - E_{i,j})^{2} = \sum_{i=1}^{m} \sum_{j=1}^{n} (O_{i,j} - a_{i} \cdot b_{j})^{2} = \min. \]

This equation leads very easy (derivatives should be null in the minimum) to a system of equations. Unfortunately, its major disadvantage is that admits infinity (a simple infinity) of solutions (i.e. for any fixed a_{i} has only one solution). Its minor disadvantage is that trying to express all other variables depending on one of them (or all depending to a parameter) leads to polynomials of degree min(m,n) without simple form in the general case. Thus one way in which the solution may be found (only in numerical case) is guessing a starting value and iterating directly from the system of equations.

Since all values are relative to one of them, starting values give only one solution (the nearest one). We choose to start with a_{i}^{0} given by following formula, and then iterate repeatedly with (b_{j}^{1}; a_{i}^{1}), (b_{j}^{2}; a_{i}^{2}), ..., until S^{2} converged:

\[ a_{i}^{0} = \sqrt{\frac{\sum_{i=1}^{m} \sum_{j=1}^{n} O_{i,j}}{r}}; \left\{ a_{i} = \sum_{j=1}^{n} b_{j} O_{i,j} / \sum_{j=1}^{n} b_{j}^{2}, i = 1..m; \quad b_{j} = \sum_{i=1}^{m} a_{i} O_{i,j} / \sum_{i=1}^{m} a_{i}^{2}, j = 1..n \right\} \]

The solution (rows and columns factors) is given in Table 2.

**Results and Discussion**

Tab.2

| row(i) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| a_{i}  | 5.637 | 5.3042 | 4.6295 | 4.832 | 4.2522 | 3.7921 | 3.7769 | 3.4937 | 3.2801 |

| column(j) | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| b_{j}     | 4.4859 | 4.2914 | 3.1425 | 2.8649 | 2.3001 | 3.7666 | 3.9267 | 2.6094 | 6.5856 | 5.5636 |
A distribution analysis can be conducted on the whole data from Table 1, which passed the independence test. By taking into account that the data are integers only, a suitable distribution is a discrete one. Table 3 contains the analysis with discrete type distribution alternatives.

| Distribution Parameters | K-S p  | A-D p  | C-S p  |
|-------------------------|--------|--------|--------|
| Uniform                 | a=6 b=28 | 0.13043 | 22.35  | 9.7·10⁻⁸ |
| Geometric               | p=0.05525 | 0.40041 | 2.8·10⁻¹⁷ | 17.783 | 6.7·10⁻⁷ |
| Logarithmic             | θ=0.98663 | 0.60571 | 0      | 41.427 | 0      |
| Neg. Binomial           | r=11 p=0.609 | 0.08675 | 0.481  | 0.80817 | 0.408  | 1.63   | 0.443 |
| Poisson                 | λ=7.1  | 0.18105 | 4.7·10⁻¹⁰ | 10.152 | 4.8·10⁻⁷ |

Results given in Table 3 clearly indicate that the distribution of the antibacterial activity of essential oils extracts among bacteria is of negative binomial type. A mathematical analysis of the negative binomial distribution allows explaining of this fact. Thus, a simple math gives:

$\text{NegBin}(x; r, p) = \int_0^\infty \text{Poisson}(x; z) \cdot \text{Gamma}(z; r, \frac{p}{1-p})dz$

where

$\text{NegBin}(x; r, p) = \frac{\Gamma(r + x)p^r(1 - p)^x}{\Gamma(x + 1)\Gamma(r)}, \quad \text{Gamma}(z; \alpha, \beta) = \frac{z^{\alpha-1}e^{-z/\beta}}{\beta^\alpha\Gamma(\alpha)}, \quad \text{Poisson}(x; \lambda) = \frac{\lambda^x e^{-\lambda}}{x!}$

The previous formula shows that the negative binomial distribution may arise as a continuous mixture of Poisson distributions where the mixing distribution of the Poisson rate is a gamma distribution. Thus, under these assumptions that it should be behind of this distribution a mixture of Poisson and Gamma distributions, between parameters should be the previous proved formula. Indeed, the data behave this property. Any row and any column agree with a certain Poisson distribution (Table 4).

| Species       | Data | MLE | p  | C-S p  |
|---------------|------|-----|----|--------|
| M. flavus     | 25; 25; 19; 16; 13; 22; 23; 15; 35; 30 | -33.384 | 22.6 | 0.96235 |
| B. subtilis   | 24; 22; 18; 18; 12; 20; 22; 14; 34; 28 | -33.052 | 21.2 | 0.97719 |
| S. epidermidis| 20; 20; 16; 14; 10; 18; 18; 12; 32; 28 | -34.557 | 19.0 | 0.84516 |
| S. enteritidis| 20; 20; 13; 10; 9; 16; 18; 10; 27; 24 | -33.470 | 16.7 | 0.51709 |
| S. typhimurium| 18; 17; 11; 8; 8; 16; 16; 10; 25; 20 | -31.897 | 14.9 | 0.37285 |
| E. coli       | 16; 16; 12; 9; 9; 14; 14; 10; 26; 22 | -31.350 | 14.8 | 0.884  |
| E. cloacae    | 14; 14; 9; 9; 12; 12; 10; 25; 22 | -31.372 | 13.6 | 0.74308 |
| L. monocytogenes| 16; 13; 9; 8; 8; 10; 11; 9; 25; 18 | -31.259 | 12.7 | 0.63353 |
| M. s.         | 25; 24; 20; 22; 20; 18; 16; 14; 16 | -24.538 | 19.444 | 0.93832 |
| M. p.         | 25; 22; 20; 20; 17; 16; 14; 13 | -24.633 | 18.556 | 0.59374 |
| C. I.         | 19; 18; 14; 16; 13; 11; 12; 9; 9 | -23.817 | 13.444 | 0.98545 |
| C. a.         | 19; 18; 14; 14; 10; 8; 9; 9 | -25.167 | 12.111 | 0.69926 |
| M. c.         | 13; 12; 10; 9; 8; 9; 8 | -20.013 | 10 | 0.21701 |
| L. a.         | 22; 20; 18; 16; 16; 14; 12; 10 | -24.412 | 16.222 | 0.74614 |
| O. b.         | 23; 22; 18; 18; 16; 14; 12; 11 | -24.965 | 16.889 | 0.69564 |
| S. o.         | 15; 14; 12; 10; 10; 10; 10; 10 | -20.654 | 11.333 | 0.28949 |
| O. v.         | 35; 34; 30; 32; 27; 25; 26; 22; 25 | -23.626 | 28.778 | 0.41834 |
| T. v.         | 30; 28; 26; 28; 24; 20; 22; 22; 18 | -25.333 | 22.422 | 0.9651 |

$C-S=ln(1/p_{C-S})+ln(1/p_{A-D}); \Sigma ln(1/p_{C-S})=12.3; p_{C-S}="Poisson" = 0.8741$
Results obtained so far show that two parts out of three results directly from the analysis of the distribution of observed data (Negative Binomial distribution of the whole pool of independent data; Poisson distribution of the series of independent data). More, let's note that with the data from Table 4, Average(λ) for Bacteria is 17.1000 and Average(λ) for Plants is 17.0999. It remains only that Poisson parameters of the series to be Gamma distributed. Indeed, results given in Table 5 proof this fact.

### Table 5

**Maximum Likelihood Estimation (MLE) of Poisson parameters of species from Table 4**

| Hypothesis | r=14.127; p=0.547 | r=9.788; p=0.636 | r=28.309; p=0.377 |
|------------|---------------------|---------------------|---------------------|
| λ_A ~ Gamma(r,p(1-p)) | | | |
| $\partial LE/\partial r=0$ | $\partial LE/\partial p=0$ | $\partial LE/\partial p=0$ | $\partial LE/\partial p=0$ |
| 10 | 0.631 | 1.710 | -55.801 | 0.993 | 0.833 | 0.917 | 0.276 | 0.964 |
| 11 | 0.609 | 1.555 | -55.561 | 0.997 | 0.878 | 0.948 | 0.187 | 0.980 |
| 12 | 0.588 | 1.425 | -55.401 | 0.999 | 0.902 | 0.974 | 0.130 | 0.988 |
| 13 | 0.568 | 1.315 | -55.310 | 0.999 | 0.909 | 0.865 | 0.241 | 0.971 |
| 14 | 0.550 | 1.221 | -55.277 | 0.998 | 0.901 | 0.846 | 0.273 | 0.965 |

### Table 6

**Different likelihood estimates for Negative Binomial distribution parameters of species**

| Hypothesis | r | p | (1-p) | MLE | p_a | p_a-D | C-S | C-S | p_C-S |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Obs ~ NegBin(r,p) | 9 | 0.655 | 1.900 | -293.474 | 0.410 | 0.366 - 1.897 | 0.387 |
| 10 | 0.631 | 1.710 | -293.137 | 0.461 | 0.398 - 1.696 | 0.428 |
| 11 | 0.609 | 1.555 | -293.001 | 0.436 | 0.372 - 1.801 | 0.406 |
| 12 | 0.588 | 1.425 | -293.008 | 0.444 | 0.372 - 1.801 | 0.406 |
| 13 | 0.568 | 1.315 | -293.120 | 0.436 | 0.337 - 1.918 | 0.383 |
| 14 | 0.550 | 1.221 | -294.755 | 0.428 | 0.297 - 2.063 | 0.357 |
| 27 | 0.388 | 0.633 | -297.695 | 0.164 | 0.035 - 5.160 | 0.076 |
| 28 | 0.379 | 0.611 | -298.034 | 0.153 | 0.030 - 5.384 | 0.068 |
| 29 | 0.371 | 0.590 | -298.366 | 0.141 | 0.026 - 5.609 | 0.061 |
| 30 | 0.363 | 0.570 | -298.692 | 0.131 | 0.023 - 5.805 | 0.055 |
| 31 | 0.356 | 0.522 | -299.013 | 0.122 | 0.020 - 6.016 | 0.049 |
| 32 | 0.348 | 0.534 | -299.322 | 0.113 | 0.018 - 6.198 | 0.045 |

(M): estimate of p remains the same, and thus (r,p) pair is a MLE estimate for the given r
An important remark opens a discussion here. Thus, at least one out of the two individual series - the Bacteria series - is rejected to provide reasonable likelihood estimates from its Poisson parameters (Table 6, \( r \) from 27 to 32, MLE estimate of \( r \) from \( \lambda_B \) being 28). This fact excludes the opposite alternative from symmetry reasons - accepting just one alternative it means that the homogeneity hypothesis should be rejected too, which is not an acceptable result, because were proofed previously that the independence hypothesis cannot be rejected and test of independence is equivalent with test of homogeneity when Chi-Square test are involved, and it were involved. It remains that both individual series should be rejected from the simultaneous agreement \( \text{Obs} \sim \text{NegBin}(r,p) \) and \( \lambda_B \text{ or } p \sim \text{Gamma}(r,p/(1/p)) \).

Even more, a simple calculus of the MLE estimates of \( p \) from \( \lambda_B \sim \text{Gamma}(r_B,p_B/(1-p_B)) \) and \( \lambda_P \sim \text{Gamma}(r_P,p_P/(1-p_P)) \) - values in Table 5 - gives \( p_B + p_P = 0.379 + 0.631 = 1.01 \sim 1.00 \) which is more than a coincidence, because the data behind \( \lambda_B \) and \( \lambda_P \) estimates are not independent (are the same) and thus the relationship \( p_B + p_P = 1.0 \) should be considered when estimates of the \( r_B \) and \( r_P \) are made. Consequential, the estimates from \( \lambda_A \sim \text{Gamma}(r_A,p_A/(1-p_A)) \) and \( \lambda_B \sim \text{Gamma}(r_B,q) \), \( \lambda_P \sim \text{Gamma}(r_P,1/q) \) should be linked together, and indeed, the values of \( p_A, r_A \) and their associated statistics from Table 5 and the values of \( p \) and \( r \) and their associated statistics from Table 6 sustain this hypothesis. The Table 7 contains the estimates using these relationships.

Interpreting results given in Table 7, is no reason to reject the hypotheses that between Gamma distribution parameters of Poisson estimates of the antibacterial activities and Negative Binomial distribution of the observables it exists the relationship given by the convolution of the Poisson distribution and Gamma distribution:

\[
\text{NegBin}(x;r_A,p_A) = \int_0^{\infty} \text{Poisson}(x;\lambda_A) \cdot \text{Gamma}(\lambda_A; r_A, \frac{p_A}{1-p_A}) d\lambda_A
\]

and the Gamma distribution probably occurs and characterize the interaction between these two types of organisms: plants and bacteria.

On another hand, the relationship between proportions from Gamma distribution of the Poisson parameters of the bacteria and plant series of data clearly indicate that the two factors - "bacteria factor" and "plant factor" in antibacterial activity has multiplicative and complementary effect and the separation of factors given in Table 2 has statistical sustainability. This fact opens the path to construct population factors of bacteria and plants at contingency of effects in antibacterial activity. More than that, the convolution of the two distributions, Poisson and Gamma strongly suggests that the Gamma distribution occurs due to the continuous effect of factors (as values from Table 1 are). Next table contains the parameters of the Gamma distributions of the population factors.
Distributions of the population factors for plants and bacteria on antibacterial activity

| Population | Distribution | r   | q   | q/(1-q) | MLE  | pK-S | pA-D | pC-S | C-S | pC-S | b_i |
|------------|--------------|-----|-----|---------|------|------|------|------|-----|------|-----|
| Bacteria   | a_i ~ Gamma(r_{B}, q_{B}/(1-q_{B})) | 31.663 | 0.120 | 0.137 | -10.323 | 0.816 | 0.792 | -0.44 | 0.804 | 1.148 |
| Plants     | b_i ~ Gamma(r_{P}, q_{P}/(1-q_{P})) | 10.082 | 0.282 | 0.392 | -16.043 | 0.993 | 0.852 | 0.898 | 0.27 | 0.965 | 1.604 |

Following figure (Figure 1) depicts the population factors distribution of plants (FP) and of bacteria (FB) and the true distribution of the antibacterial activity as convolution of these two (AA). Let's note that the convolution of two Gamma distributions only in very rare cases has a close form (expressed by a explicit distribution function) and here is not the case. It only may be approximated with another Gamma distribution.

As can be seen from the above figure, interesting extracted information is that the bacteria have a more slim distribution than the plants have. More, all three distributions are asymmetrical with more weight to low values (low effects, low interactions are more often between them).

Conclusions

The analysis of factors conducted in this study on antibacterial activity of essential oils extracts from a series of plants on a series of bacteria revealed that the Negative Binomial distribution of the antibacterial activity is a mixture (convolution) of Poisson and Gamma distributions from which only Gamma distribution can (and should) be assigned to plant and bacteria factors expressed in the antibacterial activity. Decomposition of factors under the multiplicative effect revealed a very good agreement between observed and expected values (probability of wrong model less than 0.001). Shaping of the Gamma distribution of the factors (on a relative scale) revealed that low factor values are often than high ones (left weighting of both factors distributions). The differential entropy (which is directly linked with
population diversity) of plant factors are 40% higher than the differential entropy of bacteria, giving an estimate of over 50% higher diversity of plant factors than bacteria factors.

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