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SOME ASPECTS OF BIOASSAYS AND FIELLERS THEOREM

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ABSTRACT. In this paper we discusses on fundamental of bioassay and Fiellers theorem which is an alternative method to calculate the confidence interval of a ratio. Most of the Statistical methods deals with sums and differences of the parameters, variables but not limited to ratios, percentages. We discussed on the structure and types of Bioassays and theoretical approach of Fiellers theorem. It is used to construct the confidence intervals, providing alternatives to the exact asymmetric confidence intervals. Also discusses on two analogous of Fiellers theorem. It is a fundamental formula of statistics in bioassay. Relative potency is a term used in bioassay to refer to the ability of a test sample, of unknown potency, to produce the desired response compared to a reference sample, when tested under the same conditions.

1. INTRODUCTION

Biological assays are the methods for estimating the potency or Intensity or Efficiency of a material (or a process) by means of the reaction that follows its application to living matter. A assay can be considered as a comparative biological experiment, but the interest lies in comparing the potencies of treatments on an agreed scale instead of in comparing the magnitudes of the effects of different treatments. Biological assay or Bioassay is still regarded as a recent development in scientific method. The scientific history of bioassays began at

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the end of the 19^{th} century with Ehrlich's investigations into the standardisation of Diphtheria Antitoxin. A valuable paper by J. H. Bum (1930) emphasized the importance of Bioassays, the theory and practice of Bioassays. Later the theory and practice of Bioassays was discussed by C. I. Bliss (1947, 1950) and MC. K. Cattell (1943) and others in a series of papers. A systematic account of statistical principles for Bioassays was given by D. J. Finney (1947), C. W. Emmens (1948) published the first book devoted purely to the statistical aspects of Biological assays. For further reference see [1-13].

1.1. **Objectives.**

(1) To explain the fundamentals concepts of Bioassays.
(2) To discuss the structure and various types of Bioassays.
(3) To impart the theoretical aspect of Fiellers theorem.
(4) To enhance the wide applications of Fiellers theorem in biostatistics.

2. Preliminaries

2.1. **Structure Of Biological Assay.** The typical bioassay involves a stimulus (a vitamin, a drug, a fungicide) applied to a subject (an animal, a piece of animal tissue, a plant, a bacterial culture). The intensity of the stimulus may be varied by the investigator by giving dose to the subject and this dose can be measured (perhaps as weight, volume, concentration). Application of the stimulus is followed by a change in some measurable characteristic of the subject. This magnitude of the change depends on the dose. A measurement of this characteristic is called Response of the subject. The relationship between dose and response will not be exact but will be varied by random variations between replicate subjects. This relationship can be used to indicate the potency of a dose from knowledge of the responses. Bioassays are usually comparative, the estimate of potency being obtained relative to a standard preparation of the stimulus. For most assays, the standard preparation is likely to be a sample of either the International standard or a more readily available working standard whose potency relative to the International standard was previously evaluated.

2.2. **Types Of Bioassays.** Bioassays may be either qualitative assays or quantitative assays. For the purpose the estimation of potencies of material on living matter. Bioassays can be divided into mainly three types namely,

(i) Direct Assays,
(ii) Indirect Assays based upon a quantitative responses,
(iii) Indirect Assays based upon Quantal (All-or-nothing) responses.

2.3. **Nature Of Direct Bioassays.** The principle of a direct bioassay is that the doses of the standard and test preparations sufficient to produce a specified response, are directly measured. The ratio between these doses estimates the potency of test preparation relative to the standard preparation of the stimulus. If Z_s and Z_T are doses of standard and test preparations producing the same effect, then the relative potency ρ is given by

$$\rho = \frac{Z_s}{Z_T} .$$

Thus, in such assays, the response must be clear-cut and easily recognized, and exact dose can be measured without time lag or any other difficulty. An assay with two preparations which have a common (same) effective ingredient which produces the response such assays are called "Analytical Dilution Assays (ADAs)". An assay with two preparations which have a common effect but do not contain the same effective ingredient is called a "Comparative Dilution Assay (CDA)". Generally, the ratio of mean doses of B to A, where B may be considered as standard, gives an estimate of relative potency of test preparation (A) of standards compared with standard preparation (B) of the stimulus, i.e

$$R = \frac{\bar{X_B}}{\bar{X_A}} ,$$

where $\bar{X_A}$ mean doses of test preparation of stimulus, and $\bar{X_B}$ mean doses of standard preparation of stimulus.

3. FIELLER'S THEOREM

This theorem is of great importance to the methods of statistical analysis of Bioassays, which provides Fiducial limits for estimates of relative potency. This theorem was first given in its general form by Fieller in year 1940 in JRSS, supplement. Later it was developed in year 1954 by Fieller published in JASA, series B.

Theorem 3.1. *Suppose α and β be two parameters and write $\mu = \frac{\alpha}{\beta}$. Let a and b be unbiased estimates of α and β respectively, which are linear functions of a set of observations with normally distributed errors. (For example a, b, are the sample means, differences between means or regression coefficients obtained from experimental data).*

If $Var(a) = s^2u_{11}, Var(b) = s^2u_{22}, Cov(a,b) = s^2u_{12}$, where s^2 is the Error Mean SS from the ANOVA table; u_{11}, u_{22}, u_{12} depend on the coefficients of the observations in the definitions a, b; [For example, if $a = \frac{\sum X_i}{n}$, then $u_{11} = \frac{1}{n}$; if $a = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sum(X_i - \bar{X})^2}$ then $u_{11} = \frac{1}{\sum(X_i - \bar{X})^2}$] and an estimate of μ as $m = \frac{a}{b}$ then the upper and lower fiducial limits of μ are given by

$$\left[\frac{\left[m - \frac{gu_{12}}{u_{22}}\right] \pm \frac{t_s}{b}\left[(u_{11} - 2mu_{12} + m^2u_{22}) - g\big(u_{11} - \frac{u_{12}^2}{u_{22}}\big)\right]^{\frac{1}{2}}}{1-g}\right].$$

Here t is student's t with probability density function at the chosen level of significance and $g = \frac{t^2s^2u_{22}}{b^2}$.

Proof. Given, $\mu = \dfrac{\alpha}{\beta}$,$m = \dfrac{a}{b}$, E(a)=α, $E(a) = \beta$, $V(a) = s^2u_{11}$,$V(b) = s^2u_{22}$,$Cov(a,b) = s^2u_{12}$. Consider, an expression $(a - \mu b)$,then $E(a - \mu b) \Rightarrow E(a) - \mu E(b) = 0$,

$$\Rightarrow \alpha - \mu\beta = 0 \ \left[\mu = \frac{\alpha}{\beta}\right].$$

For any μ; $[a - \mu b]$ is a linear function of observations. It is therefore normally distributed with $E(a - \mu b) = 0$ and

$$Var(a - \mu b) = V(a) + \mu^2V(b) - 2\mu Cov(a,b) = s^2(u_{11} + \mu^2u_{22} - 2\mu u_{12}).$$

Define student's t statistic as

$$t = \frac{(a - \mu b)}{\sqrt{(Var(a - \mu b)}} \Rightarrow t^2 = \frac{(a - \mu b)^2}{(Var(a - \mu b)} \Rightarrow t^2 = \frac{(a - \mu b)^2}{(s^2(u_{11} + \mu^2u_{22} - 2\mu u_{12}))}$$

$$\Rightarrow t^2s^2(u_{11} + \mu^2u_{22} - 2\mu u_{12}) = (a - \mu b)^2.$$

$H_o : (a - \mu b) = (\alpha - \mu\beta)$ may be accepted if $(a - \mu b)^2 \leq t^2s^2(u_{11} + \mu^2u_{22} - 2\mu u_{12})$. The equality sign gives a quadratic equation is μ. Let m be the solution of μ then this equation gives

$$\left[\frac{\left[m-\frac{gu_{12}}{u_{22}}\right] \pm \frac{t_s}{b}\left[(u_{11}-2mu_{12}+m^2u_{22})-g\big(u_{11}-\frac{u_{12}^2}{u_{22}}\big)\right]^{\frac{1}{2}}}{1-g}\right].$$

Here t is student's t with probability density function at the chosen level of significance and $g = \dfrac{t^2s^2u_{22}}{b^2}$. □

4. Two Analogous Of Fieller's Theorem

The two generalizations of Fieller's theorem can be derived by using two special cases of Behren's distribution.

Theorem 4.1. First Analogous of Fieller's Theorem Statement: *Suppose a and b be any two UBE's of parameters α and β respectively based on two independent random samples drawn from two normal populations with two different unknown variances, such that $m = \frac{a}{b}$ be an estimate of the ratio parameter $\mu = \dfrac{\alpha}{\beta}$.*

$$V(a) = s_1^2u_{11}, V(b) = s_2^2u_{11} \textit{ and } Cov(a,b) = 0\,.$$

The fiducial limits for the potency ratio parameter μ are given by

$$\left[\frac{m \pm \frac{d^*}{b}\left[s_1^2u_{11}(1-g)+m^2s_2^2u_{22}\right]^{\frac{1}{2}}}{(1-g)}\right] \textit{ where } g = \frac{d^{*2}s_2^2u_{22}}{b^2}\,,$$

Here d^* is the critical value of Sukhathme d-statistic which follows Behren's distribution with f_1, f_2 df and angle parameter θ is given by $\tan\theta = \dfrac{1}{\mu}\sqrt{\dfrac{s_1^2u_{11}}{s_2^2u_{22}}}$. Also f_1, f_2 are the degrees of freedom correspond to s_1^2, s_2^2 respectively.

Proof. Given a, b are the UBEs of α and β respectively such that

$$E(a) = \alpha, E(b) = \beta, V(a) = s_1^2u_{11}, V(b) = s_2^2u_{22} \text{ and } cov(a,b) = 0\,,$$

$m = \dfrac{a}{b}$ is an estimate of $\mu = \dfrac{\alpha}{\beta}$. Consider an expression $(a-\mu b)$ and its sampling distribution as

$$E(a-\mu b) = E(a) - E(b) = (\alpha - \mu\beta) = 0,\ where\ \mu = \frac{\alpha}{\beta}\,,$$

$$V(a-\mu b) = V(a) + \mu^2V(b) = s_1^2u_{11} + \mu^2s_2^2u_{22}\,.$$

To test $H_0 : \mu = \frac{\alpha}{\beta}$ or $(a-\mu b) = (\alpha-\mu\beta) = 0\,.$

The Sukhathme's d-test statistic is given by

$$d = \frac{a - \mu b}{\sqrt{(V(a - \mu b))}} \Rightarrow d = \frac{a - \mu b}{\sqrt{s_1^2 u_{11} + \mu^2 s_2^2 u_{22}}}.$$

We accept H_0 if $\frac{a-\mu b}{\sqrt{s_1^2 u_{11}+\mu^2 s_2^2 u_{22}}} \leq d^*$ or $\frac{(a-\mu b)^2}{s_1^2 u_{11}+\mu^2 s_2^2 u_{22}} \leq d^{*2}$ for equality, we have $(a - \mu b)^2 - d^{*2}[s_1^2 u_{11} + \mu^2 s_2^2 u_{22}] = 0$. Since, the above equation is Quadratic equation in μ , it has two roots. These two roots give the fiducial limits for μ.

The fiducial limits for ratio parameter μ are given by:

$$\left[\frac{m \pm \frac{d^*}{b}\left[s_1^2 u_{11}(1-g) + m^2 s_2^2 u_{22}\right]^{\frac{1}{2}}}{(1-g)}\right] \text{ where } g = \frac{d^{*2} s_2^2 u_{22}}{b^2}.$$

Here d^* is the critical value of Sukhathme d-statistic which follows Behren's distribution with f_1, f_2 df and angle parameter θ is given by $\tan\theta = \frac{1}{\mu}\sqrt{\frac{s_1^2 u_{11}}{s_2^2 u_{22}}}$.

□

Theorem 4.2. Second Analogous of Feller's Theorem Statement: *Suppose $(a_1, b_1), (a_2, b_2)$ be any two the sets of unbiased estimates for (α, β) based on two independent random samples from two normal populations with different variances. [Where (a_i, b_i)'s may be either sample means or sample regression coefficients or differences between two sample means or differences between two sample regression coefficients, based on the two independent samples of observations with normal errors].*

Let $E(a_1) = \alpha, E(b_1) = \beta, V(a_1) = s_1^2 u_{11}, V(b_1) = s_1^2 u_{22}, Cov(a_1, b_1) = s_1^2 u_{12}$; $E(a_2) = \alpha, E(b_2) = \beta, V(a_2) = s_2^2 k u_{11}, V(b_2) = s_2^2 k u_{22}, Cov(a_2, b_2) = s_2^2 k u_{12}$ and $Cov(a_1, a_2) = 0 = Cov(b_1, b_2)$, where k is known constant, u_{11}, u_{22}, u_{12} are the coefficients of observations in the definitions of (a_i, b_i)'s s_1^2 and s_2^2 be error mean sum of squares in the concerned ANOVA table with errors DF (f_1 and f_2) respectively.

By defining $\bar{a} = \frac{w_1 a_1 + w_2 a_2}{w_1 + w_2}, w_1 = \frac{1}{V(a_1)}, w_2 = \frac{1}{V(a_2)}, \bar{b} = \frac{p_1 b_1 + p_2 b_2}{p_1 + p_2}$, $p_1 = \frac{1}{V(b_1)}, p_2 = \frac{1}{V(b_2)}$ and $\bar{m} = \frac{\bar{a}}{b}$ is an estimate of $\mu = \frac{\alpha}{\beta}$; then the fiducial limits for the ratio parameter μ are given by

$$\frac{\left[m - g\frac{u_{12}}{u_{22}}\right] \pm \frac{d^* q^{\frac{-1}{2}}}{b}\left[\left(u_{11} - 2\bar{m}u_{12} + \bar{m}u_{22}\right) - g\left(u_{11} - \frac{u_{12}^2}{u_{22}}\right)\right]^{\frac{1}{2}}}{(1-g)},$$

where $\frac{d^{*2}q^{-1}u_{22}}{\bar{b}^2}$ *and* $q^{-1} = \left(\frac{1}{s_1^2}+\frac{1}{s_2^2k}\right)^{-1}$. *Here* d^* *is the critical value of Sukhathme's d-test statistic which follows Behren's distribution with* (f_1, f_2) *df and the angle parameter* θ *is given by* $\tan\theta = \frac{s_2}{s_1}\sqrt{k}$ *.*

Proof. Given $E(a_1) = \alpha, E(b_1) = \beta, V(a_1) = s_1^2u_{11}, V(b_1) = s_1^2u_{22}, Cov(a_1, b_1) = s_1^2u_{12}$; $E(a_2) = \alpha, E(b_2) = \beta, V(a_2) = s_2^2ku_{11}, V(b_2) = s_2^2ku_{22}, Cov(a_2, b_2) = s_2^2ku_{12}$ and $Cov(a_1, a_2) = 0 = Cov(b_1, b_2)$, where k is known constant. Define $\bar{a} = \frac{w_1a_1 + w_2a_2}{w_1 + w_2}$ and $\bar{b} = \frac{p_1b_1 + p_2b_2}{p_1 + p_2}$ where $w_1 = \frac{1}{V(a_1)} = \frac{1}{s_1^2u_{11}}, w_2 = \frac{1}{V(a_2)} = \frac{1}{s_2^2ku_{11}}, p_1 = \frac{1}{V(b_1)} = \frac{1}{s_1^2u_{22}}, p_2 = \frac{1}{V(b_2)} = \frac{1}{s_2^2ku_{22}}$

$$E(\bar{a}) = \frac{w_1\alpha + w_2\alpha}{w_1 + w_2} = \alpha, v(\bar{a}) = \frac{w_1^2(\frac{1}{w_1}) + w_2^2(\frac{1}{w_2})}{(w_1 + w_2)^2} = (w_1 + w_2)^{-1},$$

$$v(\bar{a}) = \left[\frac{1}{s_1^2u_{11}} + \frac{1}{s_2^2ku_{11}}\right]^{-1} = \left[\frac{1}{s_1^2} + \frac{1}{s_2^2k}\right]^{-1} u_{11} = q^{-1}u_{11}\,.$$

Also

$$E(\bar{b}) = \frac{p_1\beta + p_2\beta}{p_1 + p_2} = \beta, v(\bar{b}) = \frac{p_1^2(\frac{1}{p_1}) + p_2^2(\frac{1}{p_2})}{(p_1 + p_2)^2} = (p_1 + p_2)^{-1},$$

$$v(\bar{b}) = \left[\frac{1}{s_1^2u_{22}} + \frac{1}{s_2^2ku_{22}}\right]^{-1} = \left[\frac{1}{s_1^2} + \frac{1}{s_2^2k}\right]^{-1} u_{22} = q^{-1}u_{22}\,.$$

Similarly it can be shown that $cov(a, b) = qu_{12}$. It should be noted that the weighted average estimates $(\bar{a}, \bar{b})$ are more precise unbiased estimates for $\mu(\alpha, \beta)$ than individual unbiased estimates (a_1, b_1), and (a_2, b_2). Also $\bar{m} = \frac{\bar{a}}{\bar{b}}$ will be a better estimate for the ratio parameter $\mu = \frac{\alpha}{\beta}$ than $m_1 = \frac{a_1}{b_1}$ and $m_2 = \frac{a_2}{b_2}$.

Consider an expression $(\bar{a} - \mu\bar{b})$ and its sampling distribution as

$$E(\bar{a}-\mu\bar{b}) = \alpha-\mu\beta \textit{ and } v(\bar{a}-\mu\bar{b}) = v(\bar{a})-2\mu cov(\bar{a}, \bar{b})+\mu^2v(\bar{b}) = q^{-1}u_{11}-2\mu q^{-1}u_{12}+\mu^2q^{-1}u_{22}\,,$$

or

$$v(\bar{a} - \mu\bar{b}) = q^{-1}(u_{11} - 2\mu u_{12} + \mu^2u_{22})\,,$$

for testing $H_o : (\bar{a} - \mu\bar{b}) = (\alpha - \mu\beta) = 0, \left(\mu = \frac{\alpha}{\beta}\right)$.

The Sukhathme d-test statistic is given by

$$d = \frac{\bar{a} - \mu\bar{b}}{\sqrt{(V(\bar{a} - \mu\bar{b}))}} = \frac{\bar{a} - \mu\bar{b}}{a^{\frac{-1}{2}}(u_{11} - 2\mu u_{12} + \mu^2u_{22})^{\frac{1}{2}}}\,.$$

We accept H_o, if $\left[\frac{\bar{a}-\mu\bar{b}}{a^{\frac{-1}{2}}(u_{11}-2\mu u_{12}+\mu^2 u_{22})^{\frac{1}{2}}}\right] \leq d*$ or $(\bar{a}-\mu\bar{b})^2 \leq d^{*2}q^{-1}(u_{11} - 2\mu u_{12}+\mu^2 u_{22})$, where d^* is a critical value of Sukhathme's d-test statistic. For equality we have

$$(\bar{a}-\mu\bar{b})^2 = d^{*2}q^{-1}(u_{11}-2\mu u_{12}+\mu^2 u_{22}) ,$$

$$(\bar{a}-\mu\bar{b})^2 - d^{*2}q^{-1}(u_{11}-2\mu u_{12}+\mu^2 u_{22}) = 0 .$$

It is a quadratic form in μ it has two roots these two roots will give the fiducial limits for the ratio parameter $\mu = \frac{\alpha}{\beta}$. Thus the fiducial limits for μ are given by

$$\frac{\left[m - g\frac{u_{12}}{u_{22}}\right] \pm \frac{d^* q^{\frac{-1}{2}}}{b}\left[\left(u_{11}-2\bar{m}u_{12}+\bar{m}u_{22}\right) - g\left(u_{11}-\frac{u_{12}^2}{u_{22}}\right)\right]^{\frac{1}{2}}}{(1-g)} ,$$

where $\frac{d^{*2}q^{-1}u_{22}}{\bar{b}^2}$ and $q^{-1} = \left(\frac{1}{s_1^2}+\frac{1}{s_2^2 k}\right)^{-1}$. Here d^* is the critical value of Sukhathme's d-test statistic which follows Behren's distribution with (f_1, f_2) df and the angle parameter θ is given by $\tan\theta = \frac{s_2}{s_1}\sqrt{k}$. □

Remark 4.1.

(i) *For large value s_2^2 we have $\tan\theta = \infty$ this implies $\theta = 90^0$ in this case the Behren's distribution reduces to student's t-distribution with $f_1 DF$.*

(ii) *The usual Fieller's theorem can be obtaining by replacing by $\bar{m}$ by m, $\bar{d}$ by t_{cri}, $q^{\frac{-1}{2}}$ by S and $\bar{b}$ by b.*

5. CONCLUSION

Biostatistics may be defined as the quantitative analysis of biological phenomena based on the concurrent development of theory and observation related by appropriate methods of statistical inference. Bioassays and Quantitative Genetics can be considered as two main specialized areas of Biostatistics. The Fiellers theorem is a statistical method that can be applied to estimate the ratios and confidence intervals. Fiellers theorem is based on the assumptions that parameter estimation are Gaussian, other methods cannot be applied when only descriptive statistics is available. This theoretical approach may apply to the study of simulation, dose response, clinical trials, efficiency of drugs, standardized mortality ratio (Environmental and Occupational health), sensitivity and specificity etc. Thus Fiellers theorem can exact to obtain the asymmetric confidence intervals to the relative potency.

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