CHAPTER 7: DISCUSSION

7.1.1 Concerning The Main Study Hypotheses

The current study hypothesized the following:

1. Variances of exponential phase growth rates of *E. Coli* K-12 MG 1655 will be subject to systematic variations depending on time lag between TOFIs.

2. Rates will correlate with *Shadbala* strength of one or more *Grahas*.

3. TOFIs are valid *Muhurta* for testing predictions.

4. Eclipses influence bacterial growth processes.

Hypothesis 1 may be divided into two parts:

(a) growth processes with same TOFIs have smaller variances than those from many TOFIs;

(b) smaller variances will increase to the overall variance as time between TOFIs increases.

Hypothesis 1a was tested by F tests comparing within-column variances of **Table 6.4**'s group of 91 growth curves, and noting that they were smaller than for other columns.

Hypothesis 1b was tested by verifying the sequence of increasing within column variances from Table 6.4, columns 2 to 5, particularly Row 6 in **bold**; also F tests in the last 3 rows. Also shown in column 3 of Table 6.5, values of which are graphically displayed in Figure 6.3. **Table 6.4** compares variances within & between subgroups; to explain further and as support to graph. **Table 6.5** (is added &) shows increasing variances with varying time between TOFIs where in T.S.S. means Total Sample Size and thus **Table 6.5** gives "Within and Overall Variances for

Subgroups" being arranged for the purpose of sub-group variance analysis (and for the clarity of what is being represented graphically). High levels of statistical significance leave little room for doubt about the validity of these results.

Variations between experiments in the growth related parameters like growth rate, cell number, optical density at a given phase of growth etc. are regularly seen in microbial growth experiments. These variations are usually considered as anomalous variations and often attributed to changing ambient conditions or to small differences in the batches of growth media from one day to another, all together classified under the category of artefacts. Pioneering work was done by Rameshrao et al., who first identified this phenomenon and designed experiments to try and understand reasons for variation from Jyotisha perspective. First empirical evidence emerged from these studies to show that the variations are not completely anomalous and scientifically valid table evidence can be generated to explain variations. The current study independently validates the findings of Rameshrao et al. but further goes on to establish a complete study design and protocol, requiring very basic setup, for testing hypotheses in this emerging field of scientific.

Discoveries made in these experiments have transformed it into a study of the scientific nature of time itself, derived from the observed impact of time on exponential phase growth rates of *E. Coli* cellular growth processes. The study shows that it is possible to assess the general effects of individual *Jyotisha Grahas*, or other conditions held to be important in *Jyotisha* like eclipses, through bacterial growth experiments. to be able to do this, bacterial growth parameter (any) needed to be correlated with an empirical Jyotisha parameter. In the current study, the slope of the curve during exponential phase of growth was chosen as bacterial growth parameter and Shadbala as Jyotisha parameter. Empirical testability of Shadbala was for the first time hypothesized, designed and tested in the current study.

The innovative vision of the present growth experiments has resulted in two new major classes of discovery about biological systems. The first concerns the hitherto inexplicable variations in levels of microorganism reproduction in microbial propagation experiments. The second demonstrates a new means of quantifying the effects of individual *Jyotisha Grahas* on those reproduction levels.

7.1.1 Main Discovery 1: Two New Laws Concerning Variances in Bacterial Growth.

The exponential phase growth rates of growth curves performed over several days exhibit the typical high variations for which no valid scientific explanation has previously been offered. Dr. Rameshrao's experiments demonstrated their occurrence in other variables like final values of cell mass index (CMI) or nephelometric turbidity. Bacteria seem capricious in any measure of their growth behavior (Macansantos & Quaranta, 2014; Schwabe, 2014; Tomelleri et al, 2008; Detela et al, 2018).

In the current study, Variance in bacterial growth was assessed by comparison of groups of GCs and their mean slope values. Decision on the slope as a parameter to reflect on the differences in the growth rate (rate because we are talking about accumulation of number of bacteria over a given time) was arrived at by conducting series of pilot studies (data not presented here in this thesis) to basically determine (a) normative growth rates in different phases of growth (lag phase, exponential phase, stationary phase as shown in figure 5.2) and identify one phase which can be used in further characterization of growth as reduced or enhanced, (b) whether we can see differences in growth rates between GCs generated on different dates, and/or times, TOFIs (c) among multiple parameters calculated (Cell number, Growth Rate, Doubling time, No. of generations in a given time, No. of generations in One Hour,) from the measured OD, identify one which reflects the rate of growth. The slope of the curve during exponential phase of growth was chosen as parameter to work with.

The total of 150 GCs generated at 102 TOFIs were generated spanning a period of one year and six months. There were GCs that were done in replicates of two and three for a given TOFI on same day (Figure 6.1, Table 6.3 and Table 6.4), GCs (in replicate) with same TOFI but on different days and, GCs at a different TOFI and on different days. Statistical analysis suggested that two fundamental rules characterize variances of Exponential Phase Growth rates of microorganism: first, variances are smallest for growth processes with the same TOFI, far smaller than for the entire set of growth curves, in our case 150, generated over long experimental time periods; second, the variances for subgroups spanning increasing times increases variances. The time period for that increase being of the order of a few days, which, in our case was two days (Table 6.5).

It is commonly observed that Vaccine production runs always yield highly variable results, even when carried out under the conditions given in vaccine production manuals like those of Merck Corp. (Aiello & Moses, 2016) and OIE (World Organization for Animal Health, 2008; OIE, 2017). One of the theory put forth to explain this (Federoff & Fontana, 2002) is that such variations are caused by bottlenecks where ‘Small Numbers of Large Molecules’ are present in rate determining processes. However, the above theory **can explain neither the high variances seen between growth processes involving single flasks, nor variance reductions for sets of two or three growth curves with identical TOFIs**. In such an approach, each cell must be considered to grow independently. Variances in growth rate between cells will then be reduced by numbers of cells involved. For growth processes, that does not hold because **IF** each cell grows independently, variances must **then be** drastically reduced by the numbers involved.

Mathematical details are as follows: Cell numbers present in one unit **μL** of volume are of the order of $\mathbf{10^6}$, which is very small compared to actual numbers in a single 250 ml flask.

This will reduce standard deviations by the square root of that number, i.e. 10^{-4}, and variances by 10^{-8}. Thus, little or no residual variance will be observed between different flasks. (Rameshrao & Hankey, 2019). The high variances observed for our *E. Coli* bacterial growth curves, therefore, negate the hypothesis concerning 'Small Numbers of Large Molecules'. (Federoff & Fontana, 2002) at any rate for microbial growth experiments.

7.1.2 Main Discovery 2: Effects of Individual *Jyotisha Grahas*.

Variations in outputs from culture of bacteria/yeast/viruses is well known and accepted. Industrial examples abound, one example is the variation in vaccine production runs mentioned above. Hitherto no experimental reasons for such large variances have been given, or there is no discourse on the constituent parts/cell process that can explain variation. (Macansantos & Quaranta, 2014).

The current study endeavors to provide reasoning for these variations by correlating the differences in growth parameters to graha balas. The data types (parameters) being measured and studied here, are completely different from those of previous microbial growth experiments, even from the IAH&VB vaccine productions runs (Rao et al., 2013a). Previous experiments reported final values of cell mass index and nephelometric turbidity, as depicted in Table 7.1. Multiple production runs were carried out each day, for eight to ten days (Rao et al., 2013a).

Table 7.1: Ten Starting Time Experiments in Veterinary Microbiology (Rao et al., 2013a)							
No	System	Vaccine	Dates	DF	p value	Test	p value(s)
1.	Immune Response	PPR	02.12.07	101	0.0029	$t = 4.9$	2×10^{-6}
2.	Immune Response	PPR	09.08.08	24	3×10^{-5}	Binomial	5.6×10^{-7}
3.	Vaccine Culture	BT Virus	08.2011	4 x 4	0.0039 0.0140	Binomial	0.0002 $p_2 = 10^{-6}$
4.	Vaccine Culture	C. Chauvoie	10.2011	5 x 8	0.0001 0.0001	$F_R = 31$ $F_C = 9.4$	$<< 10^{-8}$ (overall)
5.	Vaccine Culture	Raniket Virus	18.11.11	5 x 7	$< 10^{-11}$	Inter Column	0.24×10^{-12} (overall)
6.	Vaccine Culture	P. Multocida	02.2012	5 x 7	$p_1 < 0.0001$ $p_2 = 0.001$	$F_R = 9.83$ $F_C = 4.36$	$p_t < 10^{-7}$
7.	Vaccine Culture	BT Eclipse 1	20.05.12	4 x 7	Overall <0.0003	$F_R = 11.13$ $F_C = 11.75$	p_R 0.0002
8.	Vaccine Culture	BT Eclipse 2	14.11.12	4 x 8			p_L 0.0002
9.	Vaccine Culture	BT Eclipse 3	10.05.13	4 x 5			$p_T = 4 \times 10^{-8}$
10.	Immune Response	REO Virus	10.05.13	5x30	<0.0001	$t = 3.81$	0.0006
Caption: Table 7.1 gives details of Ten Starting Time Experiments in Veterinary Microbiology							

Table 7.1 summarizes results from the previous research. It was observed that bacterial growth increased under *Guru*'s influence, and bacterial growth decreased under *Rahu'*s influence (Rao et al., 2013a). Virus propagation was oppositely affected, *Rahu* increased virus propagation rates, while *Guru* protected cells from infection (Rao et al., 2013a). A radical new finding was that a strong *Chandra* could negate effects of *Rahu*. All experiments had high levels of statistical significance: **p** being $\mathbf{p} \leq 10^{-6}$, less than one-in-a-million; for some even smaller. Over 8 experiments, significance accumulated to $\mathbf{p} \leq 10^{-65}$ (Rao et al., 2013a).

The finding that *Guru* and *Rahu* oppose each other is noteworthy: *Guru* supported life, while *Rahu* was against it as traditional texts indicate (Santhanam, 1984 Vol. I and Vol. II) and as referenced in Rameshrao & Hankey (2019). These results yield further experimental support for traditional roles of *Guru* and *Rahu*.

In this study we try to explain anomalous variations based on TOFI Jyotisha muhurtas. In order to understand the effect of *Grahas*, study is done by assessing the *Shadbala* strength of the *graha* at the TOFI, *Jyotisha muhurta* applying principles of *Jyotisha* (Santhanam, 1984). A detailed description of the Shadbala calculation is provided in Appendix -3.

Exponential phase growth rate was calculated as the slope of the line of regression spanning five data points for times 180, 210, 240, 300 and 360 minutes. The experiment also studied growth variables connected to bacterial growth, like cell numbers, doubling time computed from OD (data not shown in this thesis) which also concurred with the observations derived from comparison of slopes.

The data of the 72 growth curves generated during non-eclipse TOFIs was correlated with the Shadbala strengths of each graha and found that *Graha Kuja* (Mars) negatively impacts rate of bacterial growth. GCs done during Eclipse days are omitted for this Shadbala analysis for obvious reasons.

Like the study hypotheses for the present research (see Chapter 4), the previous research based its study-hypotheses on the traditional *Jyotisha* literature, treating statements as conjectures for experimental testing. Results offered empirical evidence that *Grahas* in *Lagna* at TOFI influence microbial growth. All studies suggested that one or more *Grahas* cause variations in rates of chemical reactions in single cells (Rameshrao & Hankey, 2019).

The inferences reported here are in agreement with these previous observations, and can therefore be attributed to the presence of the *graha* in *Lagna*, *Guru* supporting *E. Coli* growth rate, and *Rahu* opposing and decreasing it. This clearly represents a heterogeneous factor in the time dimension supporting the ‘idea’ that fundamental heterogeneity exists in the time dimension. The experiments’ success means that they demand a scientific theory of

Jyotisha: how may properties of *Grahas* influence cellular processes (Hankey, 2013). Rather than indicting molecular or microbiology, the results suggest that some external factor is exerting a coherent influence on flasks of cells with the same TOFI. They also suggest that such time-connected influences only vary slowly over time periods of up to a few days.

What subfield of biology could possibly mediate such influences? A new field with immense implications for the whole subject is complexity biology (Kauffman, 1996). The great scientist, Sir Paul Nurse, has emphasized that biology must 'get to grips' with it. (Nurse, 2014) Complexity has introduced totally new concepts to biology: fractal physiology (Bassingthwaighte et al., 1994) and the phenomenon of self-organized criticality (**SOC**). (Bak et al., 1987) **SOC** was a concept introduced to explain why a series of identical stimuli can produce highly variable responses. If the biological system is maintained at an instability, then distributions characteristic of instabilities will result, rather than those of stable systems.

Lying at the heart of biological regulation, SOC underpins fractal physiology. A large fraction of regulated physiological systems, if not all, are apparently under its influence. Their state of regulation lies at a feedback instability; it is governed by instability physics; not the physics of stable systems. Unstable systems possess high levels of internal correlations similar to those supporting the 'quantum teleportation' phenomenon: information exchange between distantly located systems (Bouwmeester et al., 1997**).**

In simple terms, this means that nature's preferred mode of regulation of complex bio systems permits their regulatory states to couple to sources of quantum correlations without regard to distance. In complexity biology, regulatory systems *can* exhibit variances dependent on external variables, just as the data from this series of experiments implies.

Reasons why such external sources can include *Jyotisha grahas* have been comprehensively given (Rameshrao & Hankey, pp. 59-72). The theory applies to all phenomena under 'criticality' in complexity biology, especially phenomena with the large variances observed in fractal physiology. All such variances may depend on starting time variables, as seen in the present experiments. The new phenomena therefore do not lie outside science's present body of knowledge.

Fractal physiology implies that organisms site loci of control at critical instabilities, with high internal coherence. Tiny stimuli can then produce disproportionately large differences in response. Most processes involved in cell growth are regulated in this way. Tiny external stimuli acting coherently on loci of control of a cell population can significantly influence rates of growth. **The path to an explanation for our experimental results involves heterogeneous, time-dependent influences acting on criticality-based loci of control of cellular growth processes.** This idea has a big potential. It may bring new levels of understanding to biology as a whole; to cell biology in particular.

7.1.3 Eclipse days' *E. Coli* bacterial growth experiments: In the current study Eclipses was used as conjecture to test the effect of grahas on the growth of *E.Coli*. GCs were generated during both solar and lunar eclipses with multiple TOFI for each eclipse GC. Different TOFI for each eclipse coincided with eclipse transition phases. A total of 33 GCs at 30 TOFIs were generated during the six eclipses. The observation of reduced growth during a Solar Eclipse is entirely in agreement with that of Dr. Rameshrao (Rao et al., 2014; Rameshrao & Hankey, 2019). There are four types of solar eclipses: total, partial, annual and hybrid and three types of lunar eclipses: total, partial and penumbral. The hypothesis that stronger the solar eclipse, the stronger its malefic power to oppose life manifested in the case of Rao et. al., (2014) to increase virus propagation in cell cultures, and in the current study to reduce exponential phase growth rate (Table 6.9). Similarly, for Lunar eclipses, exponential phase growth rates were lower on the days of total lunar eclipses, than on the partial lunar eclipse day.

These results confirm Dr. Rameshrao's findings for solar eclipse (Rao et al., 2014; Rameshrao & Hankey, 2019, pp. 53-58), and extend them in two ways: firstly, previous findings for cells under virus attack showed that a malefic influence increased virus propagation, i.e. acting against life. The same idea, that malefic influence act against life and cell growth, was also shown by in Rameshrao's results for the influence of Rahukala TOFI's on bacterial growth. In the current study, it was confirmed by our observations of slowed exponential phase growth for high Kuja Shadbalas and solar eclipse days. Secondly, for lunar eclipses, the two total lunar eclipses had stronger effects than the partial lunar eclipse. Interestingly, the largest decrease in exponential phase growth rate was during the total solar eclipse. Since its center was in N. America while the bacterial growth experiments detecting its influence were in South Asia, we infer that, as in earlier cases, the solar eclipse affected the bio-sphere globally (https://en.wikipedia.org/wiki/Solar_eclipse_of_August_21_2017; Centre of Totality).

Also, the very high growth rate for the partial lunar eclipse, in relation to other instances of eclipse time experiments, seems significant. This was only a small partial eclipse (γ 0.87) (https://en.wikipedia.org/wiki/Lunar_eclipse), meaning that the effects of a powerful moon, which Rameshrao et al. showed to increase growth, may hardly have been compromised. This would account for the remarkably higher relative value of exponential phase growth rate observed that day (Table 6.9).

7.2 STRENGTHS, LIMITATIONS AND FUTURE SCOPE OF THE RESEARCH

7.2.1. Strengths: This study confirmed the concept of TOFI as a valid *Jyotisha Lagna Muhurtha*. Its variance analysis of subgroups is completely new, as are the results of that analysis, which are entirely **independent of *Jyotisha* concepts**.

The idea of correlating *Grahas Shadbalas* with Exponential Phase Growth Rates establishes a means to quantify effects of *Grahas* empirically through R^2 correlations, opening the door to a whole new field of research.

Together with the previous study, which it extended, it could pioneer development of new domain in biology: Similarly, **by explaining the anomalous variations in growth rates of biological systems**, hitherto considered artefacts, it could initiate yet another new approach to biological investigation.

7.2.2. Limitations: In the present research study, the bacterial growth curve experiments were done using *E. Coli* a Prokaryote. When the results of these experiments were sent for publication in The journal “Cell”, the editor replied that they could not publish; nevertheless, they would consider if done with an organism of Eukaryote group like Saccharomyces. The main result, **narrowing of distributions had not been hypothesized and were therefore ad hoc discoveries;** they should be further investigated with a dedicated research program.

7.2.3. Future Scope: The success of this research study gives valid reason for extending the logic to other relevant biological experiments. One of the best ways for large scale testing of the concept of muhurta influencing biological process is to include this in the school curriculum and obtaining feedback. And now the scientific validation of the novel, *Jyotisha* approach should enable any teacher or researcher to predict outcomes of bacterial growth experiments to greater degrees of accuracy.