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A Study on the Effect of Food Colours on Seed Respiration of *Phaseolus Vulgaris*

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Abstract
Chemical food colours can prove to be very destructive for human health. Artificial food colours can activate behaviours like hyperactivity in children; cause hypersensitivity (allergic) reactions; damage chromosomes; or even cause thyroid or urinary bladder tumors in some cases. Present study was taken up to investigate the harmful effects of these commonly used food colors. Tartrazine and curcumin are used to give yellow color to the food. Respiration has been used as the parameter to measure the effect of food colours on living organisms through recording the amount of CO_2 emitted.

Keywords: Food colours, harmful, respiration

Introduction:
The food colour industry is evolving by the day, and there are diverse varieties of food colours ranging from lightest lemon yellow to the deepest green. But why did these food colours ever become so important? The reason probably is that we always have a picture for what we are going to eat in our mind, and colour and appearance always has a significant role in it. 30% HCl is secreted by our gastric glands when we see, smell, or think about food (**Joseph, 2012**)and mostly in this case, it is human tendency to imagine food that is appealing to them (**Burrows, 2009**). In addition, an unappealing colour automatically gives a person the signal that something is wrong with what they are eating.
On the other hand, it is also important to consider the negative aspects of the colours used. A scientific study conducted on *Brassica campestris* proved genotoxic and cytotoxic effect of sunset yellow food colouring dye (**Dwivedi and Kumar,2015**) . Present study was taken up to investigate the harmful effects of these commonly used food colors. These chemical food colours can prove to be very destructive for human health. Artificial food colours can activate behaviours like hyperactivity in children; cause hypersensitivity (allergic) reactions; damage chromosomes; or even cause thyroid or urinary bladder tumors in some cases (**Mercola,2011**) . In addition to this, the use of natural food colours can also be risky when used in excess. They can cause gastrointestinal problems; blood clotting problems; allergic reactions ; and even kidney stones in the long run (**Tadimalla,2017**). The rate of respiration in germinating seeds can be easily monitored through CO_2 probes and an accurate and reliable data can be obtained. Generally Tartrazine and curcumin are used to give yellow color to the food. Tartrazine is a yellow-coloured azo dye (a chemical compound where two nitrogen atoms join two hydrocarbon groups) which is used in a variety of food products like candy (MnMs), aerated drinks, canned foods etc. and curcumin is extracted from the rhizome of the turmeric plant and is fluorescent yellow in colour. It is extensively used as an important ingredient in Indian curries, pickles, mustard etc. Curcumin powder is sometimes also used as an

antiseptic. Although turmeric is quite beneficial to human body but the Curcumin present in the powder of rhizome of turmeric do affect respiration in animals

Respiration has been used as the parameter to measure the effect of food colours on living organisms through recording the amount of CO_2 emitted. The steps of seed respiration in plants can broadly be classified as Glycolysis, Link Reaction (Oxidative Decarboxylation), Kreb's Cycle, and Electron Transport System. CO_2 is released in the Link Reaction and Kreb's Cycle; therefore these steps are most adversely affected in the process of seed respiration due to the presence of food colours (**Peters 2014**). The presence of Tartrazine, most of all, limits the steps where the action of α-ketoglutaric acid and succinic acid is involved and inhibits the emission of $CO_2$to some extent.

Kidney Beans (*Phaseolus vulgaris*) were used in this study to expose an increasing concentration of food colours because they display quick respiration tendency and can grow in standard laboratory conditions (**Grant 2017**). A CO_2 Probe (Vernier) was used throughout the investigation. It measures the amount of CO_2 released by seeds when exposed to an increasing concentration of synthetic (chemical) and natural (organic) food colours. A CO_2 sensor can measure the amount of CO_2 in ppm and percentage with high accuracy and lesser errors once calibrated. Any mild change in respiration can be recorded through the Logger Pro Software in which the duration of data collection can be adjusted.

While going through the literature of the study by **(Reyes et al** 1996), it was observed that the researchers used the optimum concentration for food colours (that is, 0.1%). Therefore, to obtain settings for the main study, a preliminary study was performed with concentration both, below and above 0.1%. At the end of a preliminary study, the final settings for the main experiment were decided to be 0%, 0.001%, 0.01%, 0.1%, and 1%, as all these settings were producing enough volume and concentration of CO_2 that can be recorded show effect.

Objective:

The Objective of the study was to assess as to what extent does increasing concentration (0%, 0.001%, 0.01%, 0.1%, and 1%) of synthetic food colour Tartrazine ($C_{16}H_9N_4Na_3O_9S_2$) and natural food colour in Turmeric (*Curcuma longa*) – Curcumin ($C_{21}H_{20}O_6$) (%) affect rate of respiration of *Phaseolus vulgaris* (Kidney Beans) seeds measured in terms of emission of CO_2 gas (ppm) using a CO_2 Sensor

Methodology

1% solutions of food colours Tartrazine and Curcumin were made by dissolving 1 g of the respective solvent in 100 ml water using a spatula and mixing using a stirrer.10 ml of these solutions were measured using a measuring cylinder and diluted in 90 ml water to make 100 ml of 0.1% solutions of both Tartrazine and Curcumin using a measuring cylinder, a dropper, and a stirrer.Similar serial dilutions were performed to make 0.01%, 0.001%, and 0.001% solutions of both Tartrazine and Curcumin in separate beakers and duly labeled. pH buffer tablets of pH 7 were added to all the solutions (both Tartrazine and Curcumin) to avoid disturbances in the rate of respiration due to differences in level of pH. 20 g of seeds were weighed for each trial using a weighing balance and a Petri Dish (the mass of the Petri Dish were subtracted). 5 trials were set up for each setting (0.001%, 0.01%, 0.1% and 1%) by taking 15 cm^3 solution in a container and putting 20g seeds in it for each trial; making sure that all seeds are completely submerged (See Image 1 and 2). These setups were put with a time gap of 30 minutes to make the process of taking readings easier and the time after which the data recording of CO2 evolution were after fixed interval of time in all the stings and their trials are taken more accurate.

All settings and trials were kept in separately labeled containers.
Apart from these, one setting with 20g seeds soaked in 15 cm^3 of 0% solution (water) were taken as a control. Seeds in each trial were soaked for a total of 24 hours and kept in an incubator with temperature set at 25°C. Once the 24 hours are over, the rate of respiration of these seeds were measured quantified by the amount of CO_2 produced using the logger-pro CO_2 Probe. It will take approximately 8-9 hours to collect all data taking into account the Warm-up time and the calibration time of the CO_2 probe. The CO_2 probe were connected to Lab Pro Interface, connected to a laptop with Logger Pro software.

A delay till the time the CO_2 ppm readings become constant as in the atmosphere (Warm-up time) was observed. The samples of *Phaseolus vulgaris* seeds were put from the respected concentration labeled container to the sampling bottle to calculate CO_2 emission one at a time. There were another delay of 90 seconds after the CO_2 sensor is put into the sampling bottle and before the reading for that trial is actually taken. This delay is the same (constant) in every trial and can also be referred to as "Calibration Time" and therefore, is also a fixed variable. For each trial, the CO_2 emissions were calculated for 60 seconds so that the mean data of all setups and trials are successfully recorded. Equal and accurate calibration time were ensured before taking CO_2 emission readings for all trials to avoid data inconsistency.
Post data collection; it was planned that first the mean of all trials would be calculated and corrected by subtracting the CO_2 that was already present in the sampling bottle (403.6 ppm) from the calculated mean. Next, the Percentage Difference and Percentage Affectivity of the data were calculated to check whether synthetic (Tartrazine) or natural (Curcumin) food colour affects the results of rate of respiration more adversely. Graphs for the same were plotted and Trendlines, Trendline Equations, and 5% Error Bars were presented. Further, the strength and nature of correlations between increasing concentrations of Tartrazine and Curcumin Solutions with the rate of respiration were calculated using the Pearson's Correlation Coefficient test.

Image 1: Tartrazine Trials

Image 2: Curcumin Trials

Fixed Variable	Why to Fix?	How to Fix?
Temperature (°C)	Respiration is an enzymatic process. All enzymes' activity increases with temperature due to increase in collision between enzyme and respiratory substrate to occupy active site but only till an optimum level. Post this, the enzyme denatures and loses its active site as temperature is increased. Thus affect CO_2 release. So, it is important to keep temperature in the lab constant while conducting the study.	All setups were placed in incubator in darkness at 25º C
pH	Like temperature, the activity of enzymes is also pH-dependent. When the pH of the solution in which the enzyme is present is extremely high or low than optimum, the shape of the enzyme and the charge properties of the substrate would be affected, resulting in the denaturation of the enzyme when it can no longer work efficiently thereby affecting the rate of respiration in terms of CO_2 evolution and therefore our results.	By using pH buffer tablets (To fix the pH at 7, a pH 7 buffer tablet is needed).
Amount of Oxygen	During aerobic respiration oxygen is a terminal electron acceptor in electron transport system (ETS). It accepts electron and oxidizes 4^{th} protein complexes of ETS and helps in oxidation of NADH and $FADH_2$into NAD and FAD which are again used in Krebs cycle where at two places Decarboxylation takes place and CO_2 is released. Fate of Pyruvate to undergo Oxidative Decarboxylation to form Acetyl CoA and CO_2 also depends on oxygen in a cell. Therefore, a larger amount of oxygen would mean more electrons are accepted during ETS,	By using the same container of 200 cm^3 for all trials and settings to ensure the volume of available oxygen of seeds while soaking them in food colour for 24 hrs.

	positively affecting the rate of seed respiration (**Sharma etal,2012)**	
Mass of Seeds (g)	If the mass of seeds in different trial varies, the readings for rate of respiration will be directly affected as a greater mass would mean greater food material and hence a greater amount of substrates will be available to respire on and produce high concentration of CO_2.	By weighing a sample of 20g seeds for each trial and setting using a weighing scale before putting it in the solution.
Age of Seeds and source of seed	Old seeds are not as efficient in terms of all metabolic processes including respiration (and hence CO_2 production) as young seeds because older seeds have lower productivity (Viglas et al 2013). Therefore, all seeds selected need to be of the same age to prevent the interference of these differences.	By taking seeds packed by the same company(BeejIndia) on the same date.
Volume of Solution (Tartrazine and Curcumin) (cm^3)	Equal volume of solvent needs to be supplied to the seeds so that the respiratory enzyme systems in seeds are equally activated. A larger volume would cause flood conditions preventing the seeds from receiving oxygen for respiration (Insausti et al 1995).	By measuring a 15ml of the solution for each trial and setting with a measuring cylinder and a dropper before using it for the study.
CO_2 Sensor (Vernier) (± 100 ppm^1)	To keep uncertainties constant so that calibration is not done again and again.	By using the same CO_2 sensor for all setting and trials.
Weighing Balance (Wensar) (± 50 mg)	To keep uncertainties constant so that calibration is not done again and again.	By using the same weighing balance for all settings and trials.
Seed soaking time and Standby Time for the CO_2 sensor	In all the setups and their trials seeds are soaked for 24 hrs to make sure that natural and synthetic food colours get enough time to enter in deepest cells of seeds and might affect respiration measured in terms of CO2 released. Standby time help sensor to better calibrated, so that the sensor gets an equal amount of time to adjust to the environmental CO_2 levels.	By keeping the CO_2 sensor in the sampling bottle before starting the readings for the same amount of time.

Observation:

Qualitative data - It was observed that in Curcumin solutions, in higher concentrations, the seeds were very turgid (more swollen) and strong odor. Obviously, they even acquire a stronger yellow colour in these higher concentrations. In higher volumes of Tartrazine, on the other hand, there were no signs of a strong or even a mild smell. However, they too showed greater turgidity at higher concentrations and again, a brighter yellow colour compared to the lower concentrations. None of the seed showed visible sign of germination with plumule and radicle.

Quantitative data - Five trials were made for all the settings (0.001%, 0.01%, 0.1%, and 1%). In addition to these, one setting of *Phaseolus vulgaris* (kidney beans) seeds was kept in the same volume of water as a control to take readings in regular conditions (no food colours added). The raw data of CO_2 emission of Tartrazine and Curcumin solutions, as collected, can be seen in the Tables below (Table 1 and Table 2 respectively), along with the corrected mean, that is calculated by subtracting the amount of CO_2 in an empty Sampling Bottle, to make up for the CO_2 in the empty space during data collection.

Concentration (%)	CO_2 Emission (± 100 ppm)					Mean (ppm)	Corrected Mean (Mean - Atmospheric CO_2)	Standard Deviation
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
0%	2525	2525	2525	2525	2525	2525.0	2121.4	0.000
0.001%	2446	2450	2480	2475	2471	2464.4	2060.8	15.372
0.01%	2386	2343	2371	2375	2360	2367.0	1963.4	16.325
0.1%	2070	2041	2034	2050	2074	2053.8	1650.2	17.612
1%	1855	1851	1890	1886	1886	1873.6	1470.0	18.929

***Table* 1: Effect of increasing concentration of Tartrazine on CO_2 emission (ppm) in *Phaseolus vulgaris* seeds**

Concentration (%)	CO_2 Emission (± 100 ppm)					Mean (ppm)	Corrected Mean (Mean Atmospheric CO_2)	Standard Deviation
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
0%	2525	2525	2525	2525	2525	2525.0	2121.4	0.000
0.001%	2480	2469	2489	2467	2485	2478.0	2074.4	9.695
0.01%	2376	2361	2389	2386	2373	2377.0	1973.4	11.158
0.1%	2265	2270	2251	2253	2241	2256.0	1852.4	11.576
1%	2135	2166	2163	2130	2131	2145.0	1741.4	17.930

***Table* 2: Effect of increasing concentration of Curcumin on CO_2 emission (ppm) in *Phaseolus vulgaris* seeds**

Percentage Difference and Percentage Affectivity - The data obtained in the study from both the independent variables was compared with the control setting by calculating the Percentage Difference (%) of the Mean CO_2 emission (PPM). This Percentage Difference is calculated using the formula below:

$$Percentage\ Difference\ (\%) = \frac{Final\ Value\ (ppm) - Initial\ Value\ (ppm)}{Initial\ Value\ (ppm)}\ X\ 100$$

Here, 'Final' is the value being compared, and 'Initial' is the value in the control setting.

Another measure used for the processing of data was Percentage Affectivity. The Percentage Affectivity is a measure indicative of how severely the independent variable affects the dependent variable. The Percentage Affectivity is calculated using the formula below:

$$Percentage\ Affectivity\ (\%) = 100 - |Percentage\ Difference|(\%)$$

Here, Difference is the Absolute value of the Percentage Difference of the corresponding concentration of food colour.

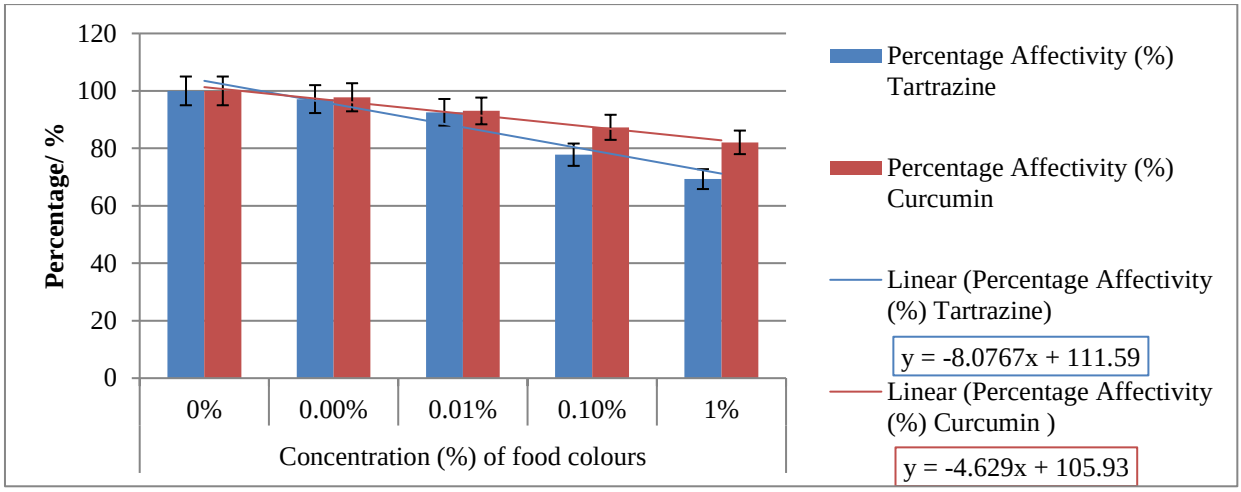
The Percentage Difference and Percentage Affectivity of Tartrazine and Curcumin solutions, with respect to the corrected mean calculated, can be seen in Table 3 and Table 4.

Concentration (%)	Percentage Difference (%)	Percentage Affectivity (%)
0%	0.000	100.000
0.001%	-2.857	97.143
0.01%	-7.448	92.552
0.1%	-22.212	77.788
1%	-30.706	69.294

***Table* 3: Percentage Difference and Percentage Affectivity of Tartrazine with regard to *Phaseolus vulgaris* seeds' respiration**

Concentration (%)	Percentage Difference (%)	Percentage Affectivity (%)
0%	0.000	100.000
0.001%	-2.216	97.784
0.01%	-6.977	93.023
0.1%	-12.680	87.320
1%	-17.913	82.087

***Table* 4: Percentage Difference and Percentage Affectivity of Curcumin with regard to *Phaseolus vulgaris* seeds' respiration**



***Graph* 1: Comparative Percentage Affectivity of Tartrazine and Curcumin on CO_2 (ppm) release regard to *Phaseolus vulgaris* seeds' respiration with 24 hrs of exposure in darkness at 25º C (error bar = 5%)**

Pearson Correlation Coefficient

The Pearson Correlation Coefficient statistical was conducted for strength and degree of correlation between increasing concentrations of food colours on CO2 released during respiration in germinating seeds. To calculate the Pearson Correlation Coefficient, the following formula is used:

$$r = \frac{\sum_i (x_1 - \bar{x})(y_1 - \bar{y})}{\sqrt{\sum_{i=1}(x_1 - \bar{x})}\sqrt{\sum_i (y_1 - \bar{y})}}$$

The "r" value (Pearson's Correlation Coefficient) of CO_2 Emission (ppm) in increasing concentration of Tartrazine solutions is -0.8199, and that in Curcumin solutions is -0.8060. This implies that both of these situations are cases of a strong negative correlation. However, since the absolute r value of increasing concentrations of Tartrazine is closer to 1, it can be concluded that it has a stronger negative correlation with CO_2 Emission (ppm) than increasing concentration of Curcumin solutions.

Results

The results showed a constant decrease in the average amount of CO_2 emission from *Phaseolus vulgaris* seeds when kept in increasing concentrations of Tartrazine and Curcumin solutions (0%, 0.001%, 0.01%, 0.1%, and 1%). The overall CO_2 Emission (adjusted with respect to CO_2 already present in the sampling bottle) of Tartrazine went down from 2121.4ppm to 1470.0ppm from 0% to 1% Tartrazine; and that of Curcumin went from 2121.4ppm to 1741.4ppm. Evidently, the concentration of Tartrazine had a more adverse effect on the respiration of the seeds. This, however, was statistically proven using several other calculations.

The calculations of Percentage Difference and Percentage Affectivity showed that even though the increasing concentrations of both, synthetic food colour (Tartrazine) and natural food colour (Curcumin) solutions worked to affect the level of seed respiration of *Phaseolus vulgaris* seeds, the synthetic food colour had a greater impact on the results since the Percentage Affectivity in this case went down from 100.000 to 69.294 %, compared to the case of natural food colour, where it went down from 100.000 to 82.087%.

It was observed that with the increase in concentration of Tartrazine on CO2 emission in *Phaseolus vulgaris* seed (kidney beans) the mean concentration of carbon dioxide was declining (Table 1) a similar trend was observed with increasing concentration of Curcumin on CO2 emission on *Phaseolus vulgaris* seeds during respiration (table 2). The standard deviation of all the data obtained in both Tartrazine and Curcumin shows uniformity. Minimum standard deviation other than control was observed in 0.001 % of Curcumin which was 9.695. At 1% Curcumin concentration the standard deviation was 17.93 which were maximum for all the concentration in Curcumin solution. Percentage difference of 2.857 % was observed as the concentration of Tartrazine increased from 0% to 0.001%. Maximum percentage difference to control was observed at 1% concentration of Tartrazine. Where the percentage difference in CO2 release is in Curcumin was maximum 17.913 % to that of control at 1%. Hence a decline in CO2 release to that of control was observed in both Tartrazine and Curcumin but a greater decrease in rate of respiration in terms of carbon dioxide release was observed in Tartrazine (Table 3 and 4).

As the entire data of carbon dioxide released in 'ppm' was converted into 100% it was quite convenient to have a comparative study of effect of Tartrazine and Curcumin on rate of respiration on *Phaseolus vulgaris* (Table 3 and 4). A Bar graph was plotted for percentage affectivity of Tartrazine and Curcumin on rate of respiration in terms of carbon dioxide released by germinating seed of *Phaseolus* with 5% error bar and linear trend line was inserted to study correlation. Overlapping of error bars was observed in data plotted in both Tartrazine and Curcumin which further represents less significant difference in the increasing concentration of food colour on respiration. As the concentration increased from 0.01 to 0.1% and later on 1 % concentration of food colour there was a significant difference in rate of respiration in terms of average carbon dioxide release by *Phaseolus* seeds. At Higher concentrations of both Tartrazine and Curcumin, that is 0.1% and 1%, it is quite evident that Tartrazine is affecting more than that of Curcumin, since the error bars (5%) of both are not overlapping. A negative regression line plotted in graph one also exhibits decline in release during respiration in germinating seeds when placed in increasing concentration of both the food colour with maximum decline was observed in Tartrazine than Curcumin, $y = -8.0767x + 111.59$ and $y = -4.629x + 105.93$ respectively. This further gives evidence to accept to alternate hypothesis.

The Pearson Correlation Coefficient test further helped us address the hypothesis since it showed that increasing concentration of Tartrazine Solution had a stronger correlation with CO_2 Emission (ppm) since its correlation coefficient was calculated to be $r = -0.8199$ compared to $r = -0.8060$ in the case of the correlation between increasing concentration of Curcumin Solution and CO_2 Emission.

Tartrazine and Curcumin both have a negative effect on the rate of respiration of *Phaseolus vulgaris* seeds. However, the effect of Tartrazine is more adverse than that of Curcumin. This investigation was conducted to see the effects chemical food colours on physiological process that might cause adverse effect to our health and to compare these effects with those of natural substances and to assess whose use out of the two will turn out to be less harmful for the consumers' health. Respiration is an important vital process that supplies energy (ATP) to cells for various functioning, like growth, development, repayment, reproduction etc.

In this study, the amount of CO_2 released by *Phaseolus vulgaris* seeds, when soaked into different concentrations of synthetic and natural food colours, was measured and compared to get an idea of how adversely these might affect a human or non human or plant test organism when taken in excessive amounts. It was found that when the seeds were soaked in synthetic food colour (Tartrazine) for 24 hours, the inhibition of the rate of respiration was greater than when the seeds were soaked in natural food colour

(Curcumin) for the same amount of time, as predicted in the alternate hypothesis before the conduction of the study. The findings of a previous study conducted by **Reyes et al (1996**), which compares the effect of different synthetic food colours on mitochondrial respiration in the kidneys and livers of rats, support the findings that we come across in our investigation. Tartrazine seems to be blocking ADP stimulated respiration in cells of kidney bean seeds also referred as 'state III respiration[2]' and hence the Electron transport system has to slow down accumulating electron released from NADH and $FADH_2$. Thus both Kreb's cycle and link step slows down releasing less CO_2.

Curcumin present in turmeric powder brings about stimulation of 'state IV respiration' (**Soto-Urquieta, et al.** 2014) In this type of mitochondrial respiration when all ADP are finally converted to ATP, respiration slows down as phosphate acceptor becomes a limiting factor. Thus similar to chemical food colour but not so intense it over all effect accumulation of NADH and $FADH_2$ produced in Krebs cycle and finally slows down release of CO_2 .A detailed study of Tartrazine on gastric mucosa of Wistar rats proved to be toxic as lymphocyte and eosinophil increased in gastric mucosa (**Moutinho, et al**,2007)

As can be seen in the tables and graphs, when the seeds are soaked in Tartrazine and Curcumin solutions of different concentrations, with an increase in concentration, the percentage difference decreases; this difference, in addition, also leads to a decrease in the percentage affectivity. A decrease in the percentage affectivity directly refers to how much food colours affect seed respiration of *Phaseolus vulgaris* seeds. The level of respiration in each studyal condition was compared to that of the control condition (0%). Therefore, the respiration of the control condition was taken to be 100% for comparison and subsequent values were compared to the control by taking out percentages. As can be seen in the tables and graphs, with increasing concentration of food colour solutions, their percentage affectivity decreases, meaning that food colours negatively affect the seed respiration of *Phaseolus vulgaris* seeds using CO_2 emission (ppm). From these observations, it can be inferred that these food colours probably have an effect on the Link Step and the Kreb's Cycle of the process of respiration of these seeds. This test therefore accepts the study's alternate hypothesis.

The Pearson's Correlation Coefficient test also demonstrated the alternate hypothesis by showing that the increasing concentrations of synthetic food colour (Tartrazine) had a stronger negative correlation with the level of seed respiration in *Phaseolus vulgaris* seeds ($r = -0.8199$) than the increasing concentrations of natural food colour (Curcumin) ($r = -0.8060$).

Discussion

The present investigation promises similar effect of increasing concentration of harmful chemical food colour azo dye Tartrazine on cellular respiration of kidney bean seeds as predicted in this piece of work previously. Any solution other than water provided to germinating seed will adversely affect respiration in seeds is well established in this investigation, as there was continuous decline in CO_2 release as the concentration of food colors increased. The kidney bean seeds appear to be a model organism in the investigation as it produced the effect to its maximum. The duration of exposure was fair enough to produce sufficient CO_2 that can be accurately detected and recorded by Vernier CO_2 sensor providing accurate and reliable results.

An important observation in the data is that the standard deviation of both, Tartrazine and Curcumin is high. However, it can be seen that the standard deviations of Curcumin settings are relatively less than

that of Tartrazine settings. This is an evidence of less reliable data because of high variability in one setting and signals that the data obtained for Curcumin settings was more reliable as compared to the Tartrazine settings. Living organism show high degree of uncertainty and respond accordingly.

While collecting the data with Vernier CO_2 sensor, warm up time brought uniformity in data obtained as all seeds in every trial were given even conditions. Mean CO_2 release of 60 seconds of data collection were carefully recorded in data table 1 and 2 for average calculation of five trials in all setups and standard deviation was calculated for the same proved to be beneficial as it brought into notice that at higher concentration of food colour cellular physiological process show higher variability. It might be due to some biochemical adduct hampering respiration.

The same scale was used to weigh the sample of seeds for the study and the same CO_2 probe was used to take the readings for CO_2 emitted by seeds to measure respiration. This ensures that the uncertainties in these readings remain constant in all settings and trials, making the readings and hence the results more reliable due to the constant nature of all the error bars.

Conclusion

It was concluded that both, synthetic and natural food colours (Tartrazine and Curcumin) had a negative effect on the respiration of *Phaseolus vulgaris* seeds. However, it was also seen that synthetic food colour (Tartrazine) had a more adverse effect on seed respiration than natural food colour (Curcumin). Since respiration is declining hence the energy required by seed to germinate and later on to grow would be affected. Both line of regression (Graph 1) and Pearson correlation (r = -ve) serves as an evidence of decline in CO_2 during cellular respiration. The extent to which decline was observed is very high in Tartrazine which is 30.706% at 1% concentration of food colour but natural food colour Curcumin of turmeric exhibited decline in respiration up to 17.913%.

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