CHAPTER 4

RESULTS AND DISCUSSION

4.1 Analysis on the Mycelium Growth Length from Different Body Part of *V. volvacea* (Inner heart and Outer surface) on Different Culture Media (MEA/PDA/NA)

In the mycelium growth length analysis, two different part of straw mushroom which were the outer surface of the mushroom Plate 4.1 and the inner middle part (heart) Plate 4.2 of the mushroom was used to culture on three different culture media which are malt extract agar (MEA), potato dextrose agar (PDA) and nutrient agar (NA). The effect of different culture media on different body part was analyzed and presented as shown in Figure 4.3, Figure 4.5, Table 4.1, Table 4.2, Table 4.4 and Table 4.5.

Based on the results shown in Figure 4.3, Figure 4.5, Table 4.1, Table 4.2, Table 4.4 and Table 4.5, MEA medium shown the best result for mycelium to fully colonize the agar plate with radial growth (8.6 cm) in 5 days for body part (outer surface) and 8 days for body part (inner heart). While, PDA medium for body part (outer surface) took additional of one day (6 days) for mycelium to fully colonized the agar plate and 10 days for body part (inner heart) to reach radial growth of 8.6 cm. NA medium shown the worst result for both body part outer surface and inner heart by not growing any mycelium at all.

The ability for MEA medium to fully colonized the agar plate with mycelium in such short duration was believed to be the easy uptake of the rich nutrients, better aeration and moisture content of the medium (Afzal *et al.*, 2013; Ghazala *et al.*, 2001). While different body part have different mycelium growth length was due to the active compound on the surface of the mushroom enable easy and faster uptake of the nutrients

in the medium to grow mycelium. As compared to the heart of the mushroom which lack of the active compound, additional time was required to absorb the nutrients to grow mycelium (Nie *et al.*, 2014) . Furthermore, NA medium does not grow any mycelium for both body part surface and heart could be due to the presence of the beef extract and peptone in the medium does not favour the growth of fungus but it is more appropriate to culture on bacteria (Nie *et al.*, 2014).

In addition, by using mathematical reasoning, the explanations above can be further supported by Table 4.3, Table 4.6, Figure 4.2 and Figure 4.4. For the body part outer surface and inner heart of *V. volvacea*, by using the equations in Figure 4.2, the radial growth of mycelium linear expansion rate, mycelium specific growth rate and doubling time for MEA, PDA and NA was able to be calculated as shown in Table 4.3.

Table 4.1: Days taken for *V. volvacea* of mycelium growth length subjected to different culture media for body part outer surface

Type of media	**Mycelium Growth Length (cm)**					
	Day1	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**
MEA	2.4±0.252	3.9±0.115	6.7±0.577	8.5±0.173	8.6±0.0	8.6±0.0
PDA	0.9±0.153	1.4±0.153	3.2±0.153	5.1±0.2	7.0±0.153	8.6±0.0
NA	0	0	0	0	0	0

Note: Values are means of 3 replicates.

Table 4.2: Days taken for full colonization for *V. volvacea* mycelium subjected to different culture media for body part outer surface

Type of media	**Days taken for fully colonization (Day)**
MEA	5
PDA	6
NA	0

Table 4.3: Kinetic study for full colonization for *V. volvacea* subjected to different culture media for body part outer surface

Type of Media	Radial Growth of Mycelium Linear Expansion Rate, k_r $(\frac{cm}{Day})$	Mycelium Specific Growth Rate, μ_w	Doubling Time, t_d (Days)
MEA	1.7033	0.4754	1.4580
PDA	1.6229	0.4529	1.5305
NA	0	0	0

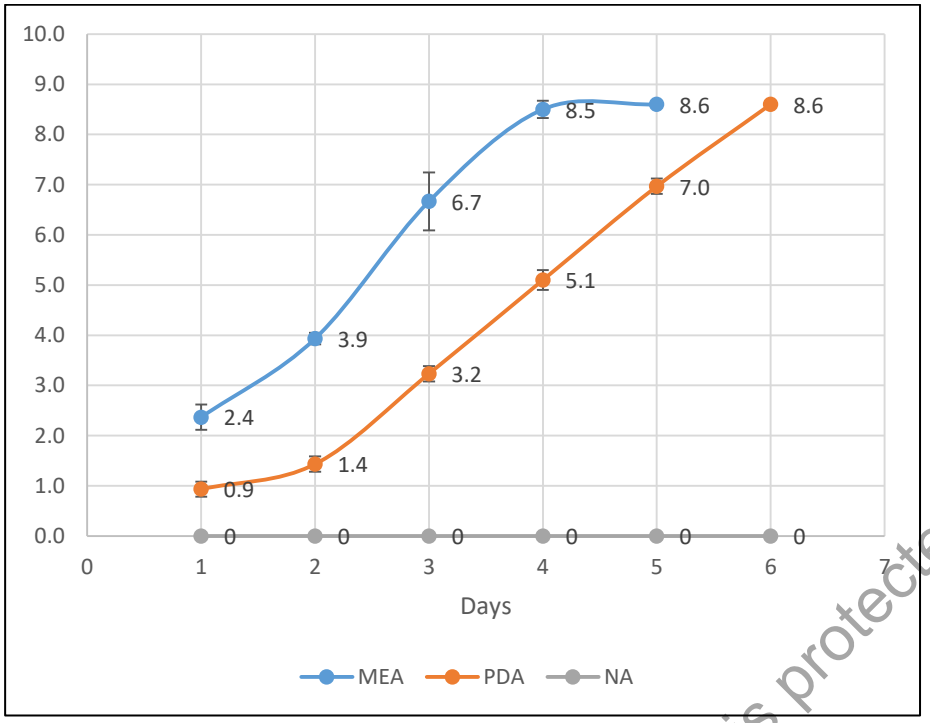

Figure 4.1: Days taken for *V. volvacea* of mycelium growth length subjected to different culture media for body part outer surface

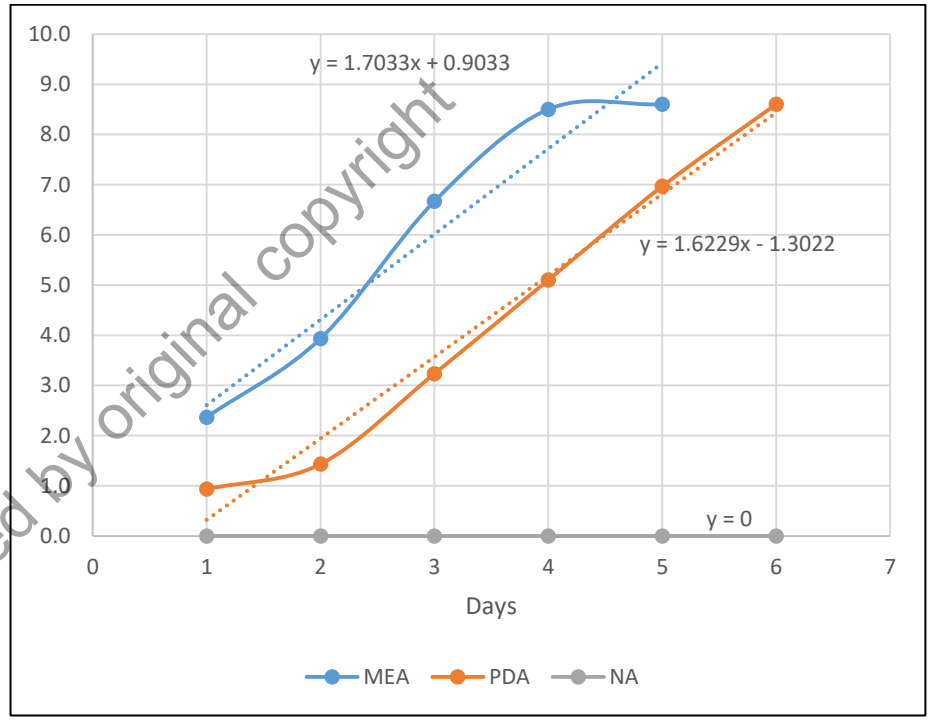

Figure 4.2: Kinetic study of mycelium growth of *V. volvacea* on different Culture Media for body part outer surface

Table 4.4: Days taken for *V. volvacea* of mycelium growth length subjected to different culture media for body part inner heart

Type of media	Mycelium Growth Length (cm)									
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
MEA	1.1± 0.1	2.1± 0.1	3.2± 0.1	4.3± 0.1	5.4± 0.252	6.6± 0.173	7.6± 0.23	8.6± 0.0	8.6± 0.0	8.6± 0.0
PDA	0.9± 0.1	1.7± 0.1	2.7± 0.2	3.4± 0.1	4.4± 0.2	5.4± 0.3	6.1± 0.1	6.9± 0.1	7.8± 0.1	8.6± 0.0
NA	0	0	0	0	0	0	0	0	0	0

Note: Values are means of 3 replicates.

Table 4.5: Days taken for full colonization for *V. volvacea* mycelium subjected to different culture media for body part inner heart

Type of media	Days taken for fully colonization (Day)
MEA	8
PDA	10
NA	0

Table 4.6: Kinetic study for full colonization for *V. volvacea* subjected to different culture media for body part inner heart

Type of Media	Radial Growth of Mycelium Linear Expansion Rate, k_r $(\frac{cm}{Day})$	Mycelium Specific Growth Rate, μ_w	Doubling Time, t_d (Days)
MEA	1.0893	0.3041	2.2795
PDA	0.862	0.2405	2.8823
NA	0	0	0

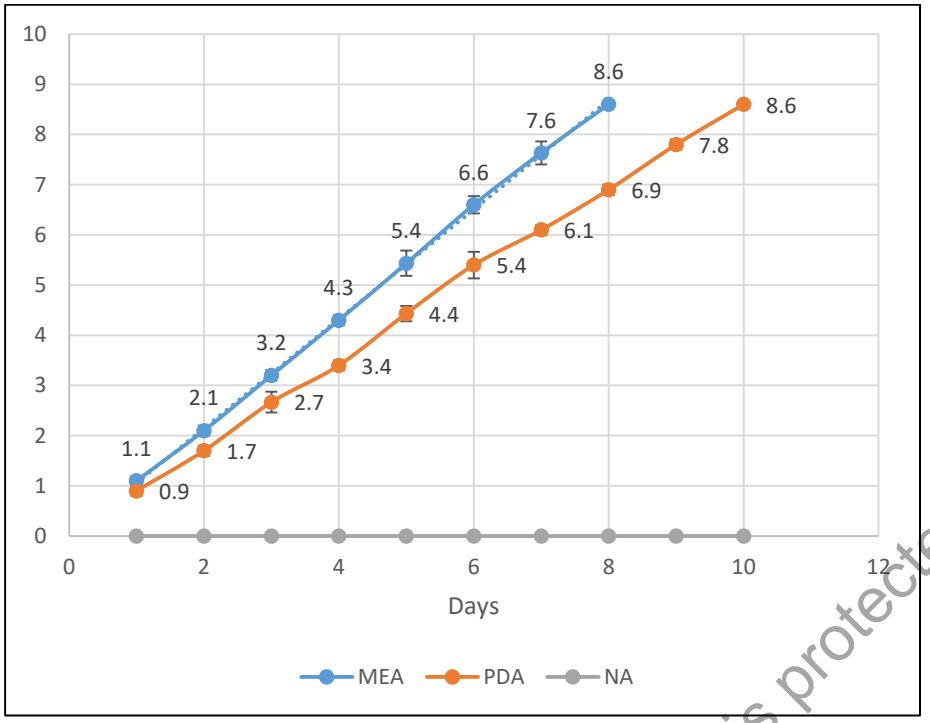

Figure 4.3: Days taken for *V. volvacea* of mycelium growth length subjected to different culture media for body part inner heart

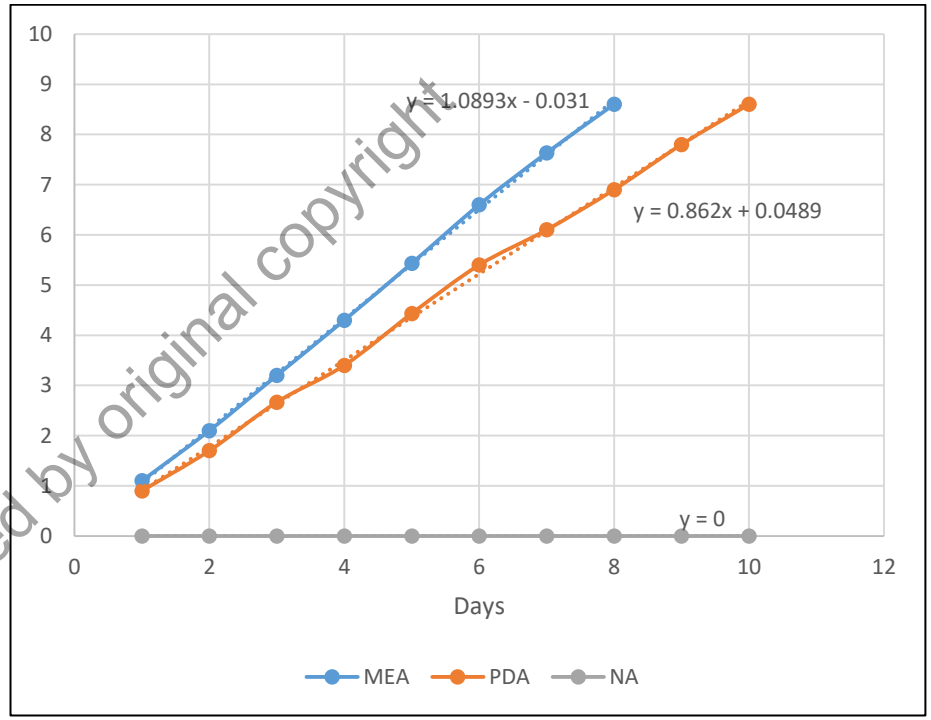

Figure 4.4: Kinetic study of mycelium growth of *V. volvacea* on different Culture Media for body part inner heart

4.2 Comparison of the Mycelium Growth of *V. volvacea* added on Composted and Non-composted EFB, Rubber-wood Sawdust and Cereal Grain (Paddy Rice and Paddy Straw) using Glass Race Tube (GRT) method

There were 5 types of different substrates which are composted and non-composted EFB, RSD, paddy rice and PS was used to carry out the spawn run test on *V. volvacea*. All these substrate are commonly found in the agricultural and manufacturing sector which were abundant and frequently discarded as a waste and will only cause negative effect to the environment and community (Kavitha *et al.*, 2013). Therefore, the purpose of this test was to recycle, reuse and find out the suitable substrate to culture the straw mushroom.

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All 5 types of the substrates was chopped and shredded into smaller size by using a shredder to reduce porosity of the substrates so that an even growth of mycelium can obtained (Kulcu, 2014). After the shredding is complete, all the substrates were soaked and immersed separately in a basket for 24 hours with distilled water instead of tap water as chlorine in the tap water would affect the growth of the spawn run and the purpose of soaking was to ensure that all the substrates was washed away from dirt and dust and absorbing maximum amount of water content for the ease of maintaining the moisture content afterwards.

After 24 hours of soaking, the distilled water was poured away and excess water was drained away by placing the substrates into an oven with 100 - 105 ˚C for 5 hours. When all the moisture was evaporated away, additional distilled water was added into the substrates to achieve 58 - 62 % of moisture content by using moisture analyzer. As studies shows that at this range of moisture content, optimum spawn run was obtained (Wang *et al.*, 2012)

On the other hand, different substrate have different capability to retain water. Thus, a careful and precise measured amount of distilled water is added into the substrates. Once the desired moisture content is obtained, five test tube is pre-weighted and filled with 5 different substrates to measure the total amount of substrates that can filled in completely the test tubes.

As soon as the actual amount of substrates after minus the weight of the test tube was attained, substrate, rice bran and calcium carbonate is added in a ratio of 200:10:1 by weight into three test tubes for each substrates. Once done, the test tubes are loosen to autoclave and let cool down for a few hours before placing *V. volvacea* spawn to culture to prevent the spawn from undergoing heat shock (Ahlawat & Tewari, 2011). After the test tubes was properly cooled down, the *V. volvacea* spawn was placed using a cork borrer and sealed with parafilm to prevent contamination and placed in 30 ˚C incubator to observe and measure.

According to Table 4.7, Table 4.8 and Figure 4.5, RSD shown the best result by colonizing 13.5 cm of the test tube within a week (7 days). Followed by paddy rice (8 days), paddy straw (11 days), non-composted EFB (12 days) and composted EFB (14 days). *V. volvacea* needs a little amount of nitrogen and much more amount of carbon to support its growth. Thus, RSD contained 0.32 % of nitrogen and 56.41 % of carbon with total carbon to nitrogen ratio of 176.28 which are the lowest nitrogen content and highest carbon and total carbon to nitrogen ratio among other substrates which proven the findings mentioned (Apetorgbor *et al.*, 2015; El-Tayeb *et al.*, 2012).

The nitrogen, carbon and total carbon to nitrogen ratio of paddy rice was treated as rice husk. Hence, the nitrogen, carbon and total carbon to nitrogen ratio of rice husk were 0.34 %, 35.13 %, 103.32 (El-Tayeb *et al.*, 2012; Abreu *et al.*, 2011). Whereas, paddy straw had a little higher nitrogen, carbon and lower total carbon to nitrogen ratio which were 0.61 %, 54.26 % and 84.0 (Rosmiza *et al.*, 2014).

The slower mycelial growth observed in non-composted EFB and composted EFB are due to the low carbon to nitrogen ratio. The composition of non-composted EFB were 0.6 % nitrogen, 44.1 % carbon and 77.7 carbon to nitrogen ratio (Ali *et al.*, 2013). Meanwhile, the composition of composted EFB were 2.8 % nitrogen, 38.5 % carbon and 13.8 carbon to nitrogen ratio (Marlina *et al.*, 2015; Razali *et al.*, 2012). Nevertheless, with this result RSD was chosen to screen and optimize the optimal parameters BBD in RSM.

In addition, by using mathematical reasoning, the explanations above can be further supported by Table 4.9 and Figure 4.6. Based on the equations in Figure 4.6, the mycelium growth rate can be calculated and as presented in Table 4.9, where the

mycelium growth rate of RSD, paddy rice, PS, non-composted EFB and composted EFB are 2.219 $\frac{cm}{Day}$., 2.0119 $\frac{cm}{Day}$.,.1.2582 $\frac{cm}{Day}$., 1.2176 $\frac{cm}{Day}$.and 1.053 $\frac{cm}{Day}$.

Table 4.7: Days taken for *V. volvacea* of mycelium growth length subjected to different substrates using GRT method

Type of Substrate	Mycelium Growth Length (cm)														
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**	**Day 15**
Paddy	0.1±	1.2±	2.9±	5.1±	7.0±	9.4±	12.0±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±
Rice	0.0	0.283	0.424	0.49	0.707	0.566	0.636	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rubber-wood Sawdust	1.3±	2.4±	5.2±	7.0±	9.8±	12.8±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±	13.5±
	0.1	0.1	1.002	1.10	1.041	0.764	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paddy Straw	0.0±	0.1±	1.4±	2.1±	2.8±	4.1±	5.6±	7.5±	9.0±	10.9±	12.2±	12.7±	13.5±	13.5±	13.5±
	0.0	0.058	0.608	0.61	0.551	0.839	1.012	1.041	2.098	2.39	2.309	1.443	0.0	0.0	0.0
Noncomposted EFB	0.05±	0.5±	0.8±	1.4±	2.8±	4.6±	6.0±	7.5±	8.8±	10.0±	11.1±	12.3±	13.5±	13.5±	13.5±
	0.071	0.424	0.424	0.4	1.061	2.828	4.243	5.657	6.718	4.95	3.394	1.697	0.0	0.0	0.0
Composted EFB	0.2	0.8	1.1	1.5	2.4	3.4	4.5	5.7	6.4	7.8	9.3	11.4	12.3	13.2	13.5
	±0.21	±0.35	±0.21	±0.0	±0.21	±0.49	±0.77	±0.91	±1.41	±1.76	±2.54	±2.334	±1.768	±0.495	±0.0

Note: Values are means of 3 replicates.

Table 4.8: Days taken for full colonization for *V. volvacea* subjected to different substrates using GRT method

Type of Substrate	Days taken for fully colonization (Day)
Rubber Wood Sawdust	7
Paddy Rice	8
Paddy Straw	11
Non-composted EFB	12
Composted EFB	14

Table 4.9: Kinetic study for full colonization for *V. volvacea* subjected to different substrates using GRT method

Type of Substrate	Mycelium Growth Rate ($\frac{cm}{Day}$)
Paddy Rice	2.0119
Rubber wood Sawdust	2.219
Paddy Straw	1.2582
Non-composted EFB	1.2176
Composted EFB	1.053

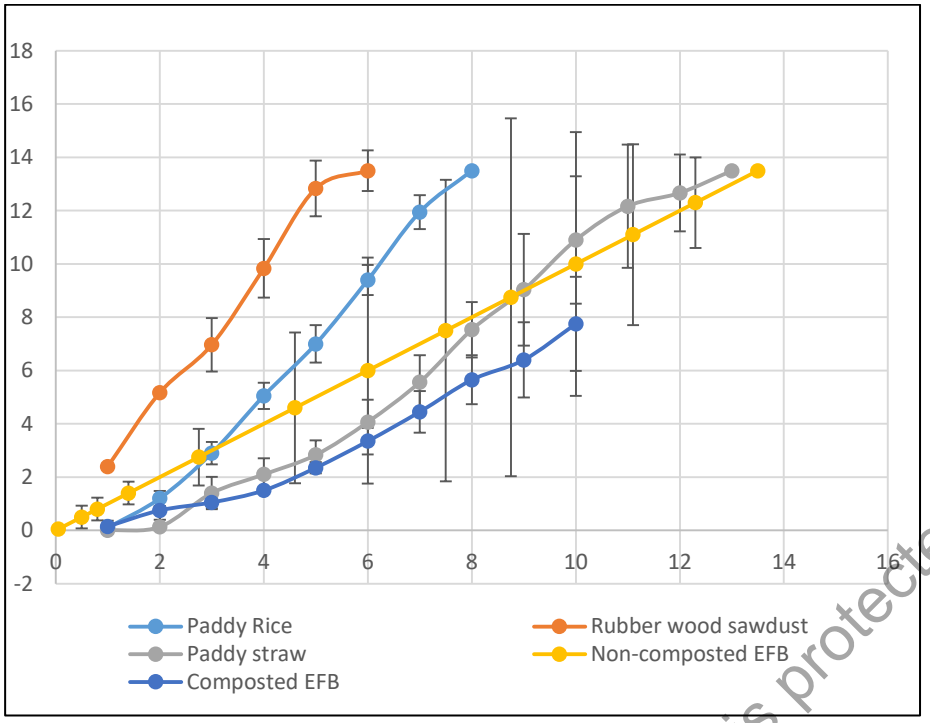

Figure 4.5: Days taken for *V. volvacea* of mycelium growth length subjected to different substrates using GRT method

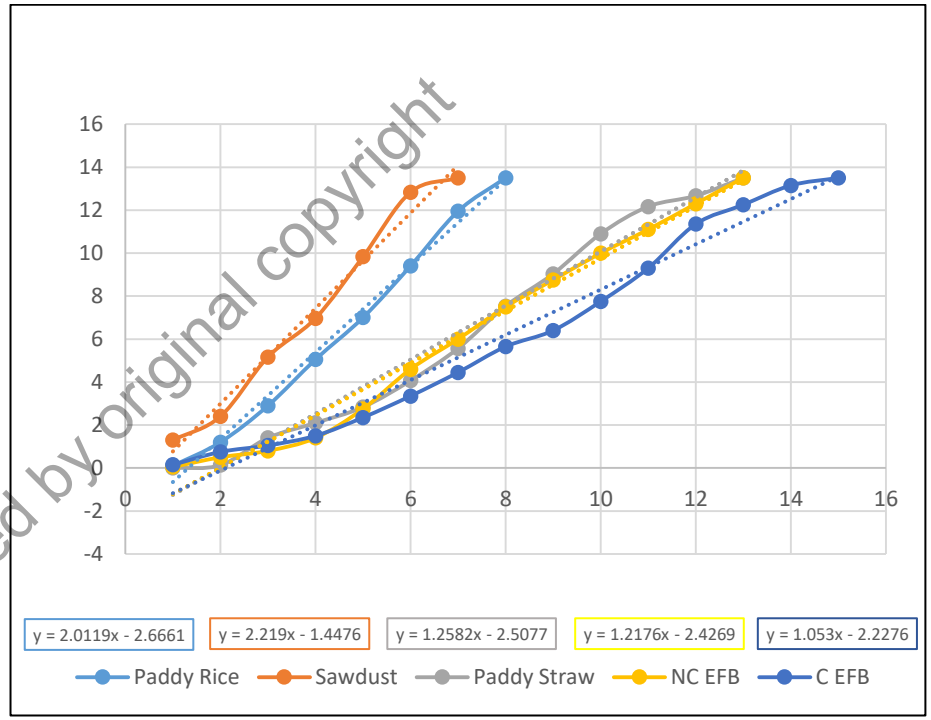

Figure 4.6: Kinetic study of mycelium growth of *V. volvacea* on different substrates using GRT Method

4.3 Optimization of the Parameters that Affect the Total Days Taken for *V. volvacea* Mycelium to Fully Colonize the Rubber Wood Sawdust Substrate by using Box-Behnken Design (BBD)

By referring to the studies of the researchers, the parameters such as temperature, amount of rice bran and amount of calcium carbonate ($CaCO_3$) played a role in influencing the growth of *V. volvacea*. Hence, in order to obtain a faster and rapid mycelium growth rate of *V. volvacea*, these parameters were being optimized. A range of values were selected and keyed in as shown in Table 3.5.

After keying in the range of values for each parameter, a set of 17 runs were suggested by the software. Each run was carried out and the results are presented in Table 4.10. This is then monitored by a careful analysis of the model.

Table 4.10: Design and response of the BBD for days taken for *V. volvacea* mycelium fully colonized RSD substrate

RUN	Factors			Response: Days taken
	(A)	(B)	(C)	Experimental
1	25.0	0.50	0.08	9
2	35.0	0.50	0.08	6
3	25.0	1.00	0.08	10
4	35.0	1.00	0.08	10
5	25.0	0.75	0.05	14
6	35.0	0.75	0.05	10
7	25.0	0.75	0.10	12
8	35.0	0.75	0.10	10
9	30.0	0.50	0.05	7
10	30.0	1.00	0.05	12
11	30.0	0.50	0.10	7
12	30.0	1.00	0.10	7
13	30.0	0.75	0.08	8
14	30.0	0.75	0.08	7
15	30.0	0.75	0.08	11

Table 4.10 continued: Design and response of the BBD for days taken for *V. volvacea* mycelium fully colonized RSD substrate

16	30.0	0.75	0.08	9
17	30.0	0.75	0.08	10

4.3.1 Statistical Analysis

The thorough design matrix along with the values of experimental response is shown in Table 4.10. A three centre points was used to link the correspondence between the RSD substrate parameters (temperature, amount of rice bran and $CaCO_3$) to the total days taken for the *V. volvacea* mycelium fully colonized the RSD substrate. There were a total of 17 run of experiments in the model design and the centre points were used to determine the experimental error.

Table 4.10 shows the result form BBD of RSD substrate composition on the total days taken for the *V. volvacea* mycelium fully colonized the RSD substrate. The minimum days taken for the *V. volvacea* mycelium fully colonized the RSD substrate was attained in run #2 (35 ˚C, 0.50 g of rice bran, 0.08 g of $CaCO_3$) which was 6 days, while the maximum days taken for the *V. volvacea* mycelium fully colonized the RSD substrate was attained in run #5 (25 ˚C, 0.75 g of rice bran, 0.05 g of $CaCO_3$) which was 14 days.

By using Stat-Ease, .Inc Design-Expert (version 10), the Sequential Model Sum of Squares, Lack of Fit Tests of the model and Model Summary Statistic was shown in Table 4.11, Table 4.12 and Table 4.13.

Table 4.11: Sequential Model Sum of Squares

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	
Mean vs Total	1487.12	1	1487.12			
Linear vs Mear	28.75	3	9.56	2.64	0.0932	
2FI vs Linear	9.50	3	3.17	0.84	0.5017	
Quadratic vs 2	25.38	3	8.46	4.83	0.0396	Suggested
Cubic vs Quad	2.25	3	0.75	0.30	0.8249	Aliased
Residual	10.00	4	2.50			
Total	1563.00	17	91.94			

From the response of the experiment shown in Table 4.11, a quadratic model was suggested by the software based on the F-value. The model's F-value and Prob > F were 4.83 and 0.0396, respectively. This implied that the model was significant. This was further confirmed by verifying the lack-of-fit tests and summary statistics of the model and the results were as follows.

Table 4.12: Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F	
Linear	37.13	9	4.13	1.65	0.3316	
2FI	27.63	6	4.61	1.84	0.2885	
Quadratic	2.25	3	0.75	0.30	0.8429	Suggested
Cubic	0.000	0				Aliased
Pure Error	10.00	4	2.50			

For the Lack of Fit Tests in Table 4.12, a model with insignificant lack-of-fit is a must. Hence, the model with p-value higher than 0.10 is chosen. The quadratic model suggested has a p-value of 0.8429, thus implying that the lack of fit was not significant and relative to the pure error.

Table 4.13: Model Summary Statistic

Source	Std. Dev	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.90	0.3789	0.2355	-0.1652	88.42	
2FI	1.94	0.5041	0.2065	-0.9868	150.76	
Quadratic	1.32	0.8386	0.6310	0.3197	51.63	Suggested
Cubic	1.58	0.8682	0.4729		+	Aliased

For the Model Summary Statistics as revealed in Table 4.13, the model with the highest Adjusted R-Squared and Predicted R-Squared is chosen. The suggested quadratic model has the highest values. Thus, this can be accepted. Next, the research was continued with Analysis of Variance for Response Surface Quadratic model (ANNOVA). Table 4.15 shows the significance of the model.

Table 4.14: ANOVA for quadratic model of days taken for *V. volvacea* mycelium fully colonized RSD substrate

Source	**Sum of squares**	**Degree of freedom**	**Mean**	**F-Value**	**p-value**	**Remarks**
Model	63.63	9	7.07	4.04	0.0396	significant
A-Temperature	10.13	1	10.13	5.79	0.0471	significant
B- Rice Bran	12.50	1	12.50	7.14	0.0319	significant
C- $CACO_3$	6.13	1	6.13	3.50	0.1036	not significant
AB	2.25	1	2.25	1.29	0.2942	not significant
AC	1.00	1	1.00	0.57	0.4744	not significant
BC	6.25	1	6.25	3.57	0.1007	not significant
A^2	9.47	1	9.47	5.41	0.0529	not significant

Table 4.14 continued: ANOVA for quadratic model of days taken for *V. volvacea* mycelium fully colonized RSD substrate

B^2	12.89	1	12.89	7.37	0.0300	significant
C^2	4.21	1	4.21	2.41	0.1648	not significant
Residual	12.25	7	1.75			
Lack of Fit	2.25	3	0.75	0.30	0.8249	not significant
Pure Error	10.00	4	2.50			
Cor Total	75.88	16				

As can be seen from Table 4.14, through ANNOVA, the adequacy of the model was further validated. Values of "Prob > F" less than 0.05 indicate that the model terms were significant. In the study, terms A, B and B^2 had a significant since each p-value was less than 0.05 effect while C, AB, AC, BC, A^2, and C^2 had no significant on the days taken for *V. volvacea* mycellium to fully colonized RSD substrate since each p-value was more than 0.05.

While Table 4.14 shows that the Fisher's test (F-value) was 4.04, which implies this model was significant. There was only a 3.96 % chance that a "Model F-Value" this large could occur due to noise. The "Lack of Fit F-value" of 0.43 implied the Lack of Fit was not significant relative to the pure error. There was a 82.49 % chance that a "Lack of Fit F-value" this large could occur due to noise. Next, is Table 4.15 shows the statistical parameters obtained from ANOVA.

Table 4.15: Statistical parameters obtained from ANOVA of days taken for *V. volvacea* mycelium fully colonized RSD substrate

Variables	Response
R^2	0.8386
Adjusted R^2	0.6310
Predicted R^2	0.3197
Standard Deviation	1.32
Coefficient of Variance (%)	14.14
Adequate Precision	8.254
Mean	9.35

The quality of the model developed was evaluated based on the correlation coefficient (R^2.) According to Table 4.15, R^2 was found to be 0.8386 and the "Pred R-Squared" of 0.3197 was not as close to the "Adj R-Squared" of 0.6310. On the other hand, the Coefficient of Variance of the model was used to describe the model fit representing the ratio of standard deviation to mean. The lower the CV, the better the model fit. The CV calculated is 14.14% which is quite low, indicating a good model fit.

Furthermore, the coefficient of full regression modeling equation and the statistical significance was being evaluated and determined. The response in Table 4.5 was obtained using multiple regression analysis and by selecting the quadratic model as recommended by the software, two mathematical models based on the experimental results Equation 4.1 (in coded factors) and Equation 4.2 (in actual factors) were derived.

Final Equation in Terms of Coded Factors:

$$Days\ taken\ for\ fully\ colonization = +9.00 - 1.12A + 1.38B - 1.00C + 0.75AB + 0.50AC - 1.50BC + 1.37A^2 - 1.63B^2 + 1.13C^2 \quad (4.1)$$

Final Equation in Terms of Actual Factors:

$$Days\ taken\ for\ fully\ colonization = +68.572 - 4.275 * Temp. + 44.5 * Rice\ Bran - 250.0 * CaCO_3 + 0.6 * Temp. * Rice\ Bran + 4.0 * Temp. * CaCO_3 - 240.0 * Rice\ Bran * CaCO_3 + 0.055 * Temp.^2 - 26.0 * Rice\ Bran^2 + 1800.0 * CaCO_3^2 \quad (4.2)$$

Where, A, B, and C were the coded values of test variables that represented temperature, amount of rice bran and amount of $CaCO_3$ respectively. The variables AB, AC, and BC represented the interaction effects of temperature and amount of rice bran, temperature and amount of $CaCO_3$, amount of rice bran and amount of $CaCO_3$ respectively.

4.3.2 Confirmation of experiments and adequacy of the models

As shown in Figure 4.7, the normal percentage probability plot was essential to check the adequacy of the model. In the normal percentage probability plot, the degree of residuals deviates from the predicted values indicates the distribution and the extent of errors occurred in the model and these errors are independent of each other's (Li *et al.*, 2004).

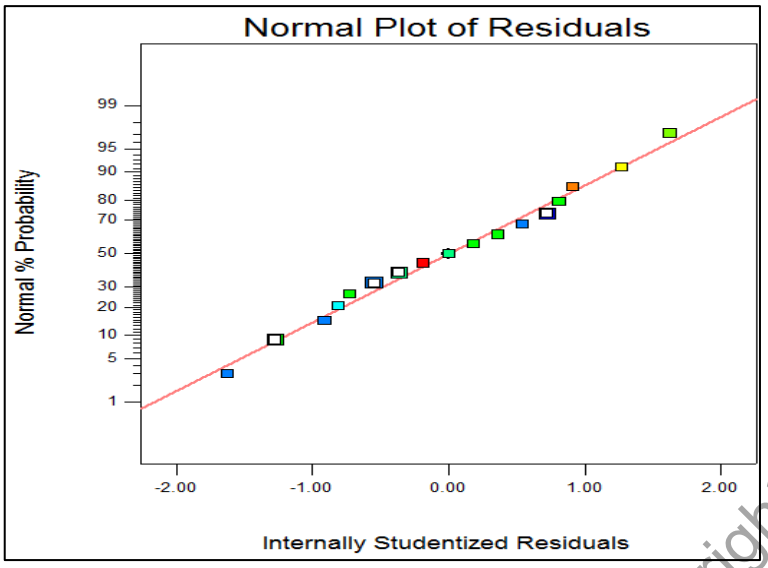

Figure 4.7: Normal probability of internally studentized residuals for days taken for *V. volvacea* mycelium fully colonized RSD substrate

Another way to check the model adequacy was by the plot of residuals versus ascending predicted response value as shown in Figure 4.8. The plot in the figure showed a random scatter of residuals across the graph. In conclusion, based on statistical ANOVA and validation of the model adequacy analysis, it was confirmed that the quadratic model fitted for the days taken for *V. volvacea* mycelium fully colonized RSD substrate.

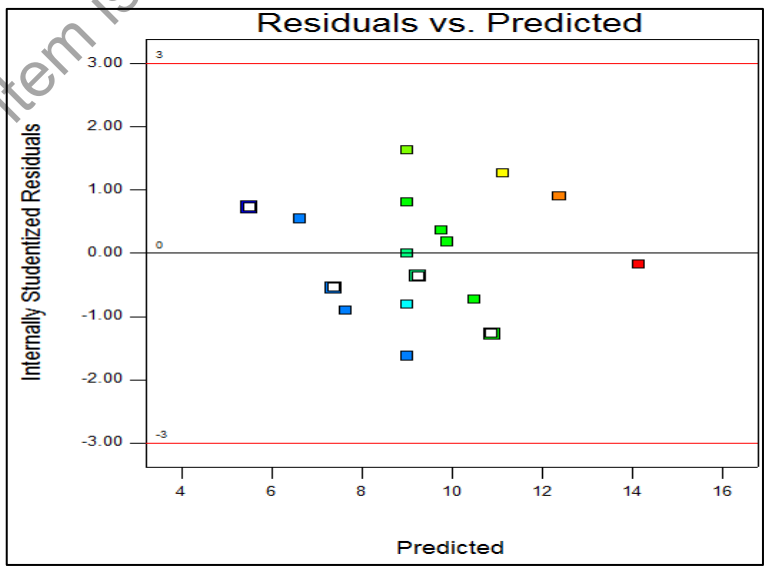

Figure 4.8: Plot of internally studentized residuals vs predicted response for the days taken for *V. volvacea* mycelium fully colonized RSD substrate

4.3.3 Model Analysis

4.3.3.1 Effect of Parameters

The results of regression analysis from Table 4.9 shows that the days taken for *V. volvacea* mycelium fully colonized RSD substrate was affected by the main factor of amount of rice bran (B) only. Temperature (A) was slightly 0.004 high from being significant, while amount of $CaCO_3$ was 0.0288 higher than 0.0500. As noted from the aforementioned table, the pretreatment study confirmed that the effect of the amount of rice bran ($p<0.05$) was higher compared to the temperature and amount of $CaCO_3$ with p-value of 0.0540 and 0.0788 respectively.

4.3.3.2 Interaction between parameters

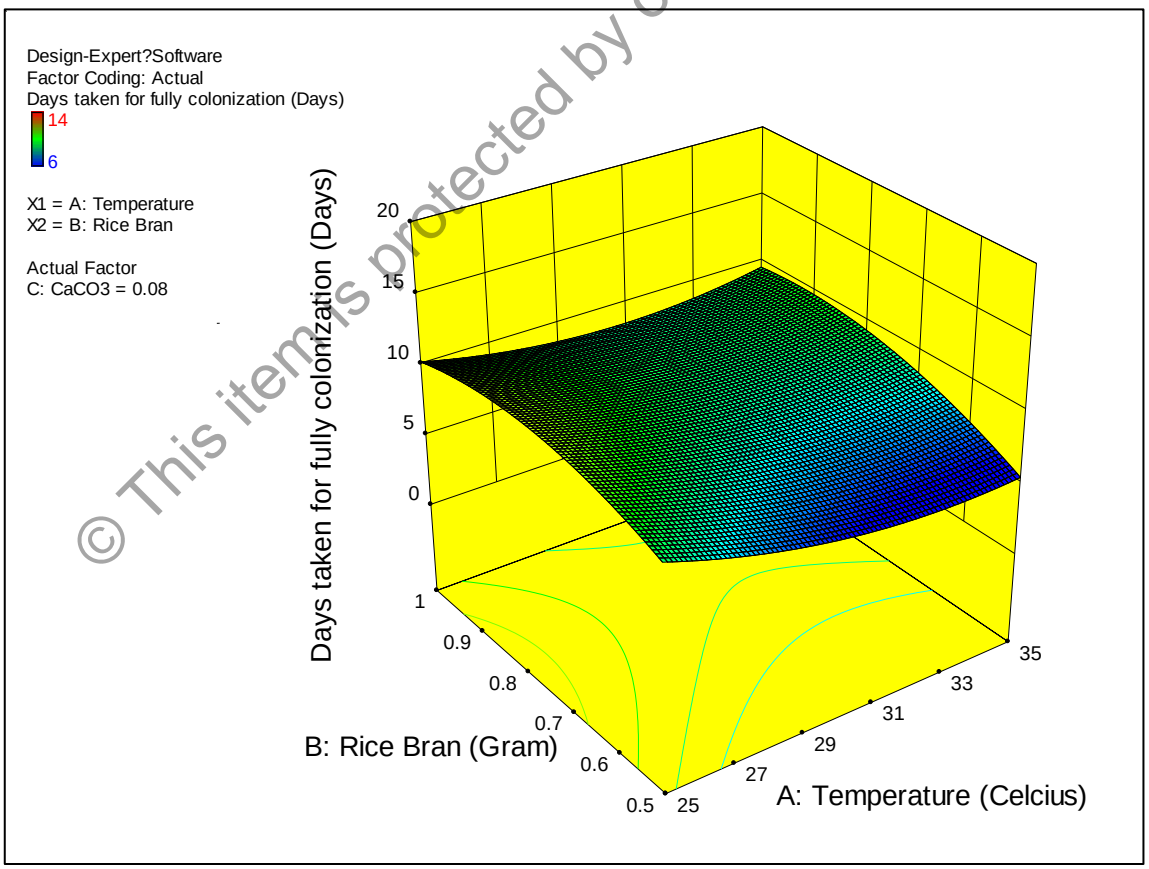

Figure 4.9: Three dimensional response surface plot indicating the interaction between temperature and amount of rice bran on days taken for *V. volvacea* mycelium fully colonization on RSD substrate

Figure 4.9 shows the three dimensional response surface plot indicating the interaction between temperature and amount of rice bran on days taken for *V. volvacea* mycelium fully colonization on RSD substrate. Based on the figure, an increases in the temperature and decreasing in the rice bran resulted in the minimum days taken for *V. volvacea* mycelium fully colonization on RSD substrate until an optimum condition was attained at 35 ˚C and 0.50 gram of rice bran. Further increased in the condition showed unfavorable effects on the days taken for *V. volvacea* mycelium fully colonization on RSD substrate. Although rice bran was an essential nitrogen source to aid on the formation of fruiting body, but there must be a balance or optimum condition when it comes to initiating the mycelium (Marlina *et al.*, 2015), therefore this was seen on the result where increasing rice bran is unfavorable during high temperature (Miles & Chang, 2004).

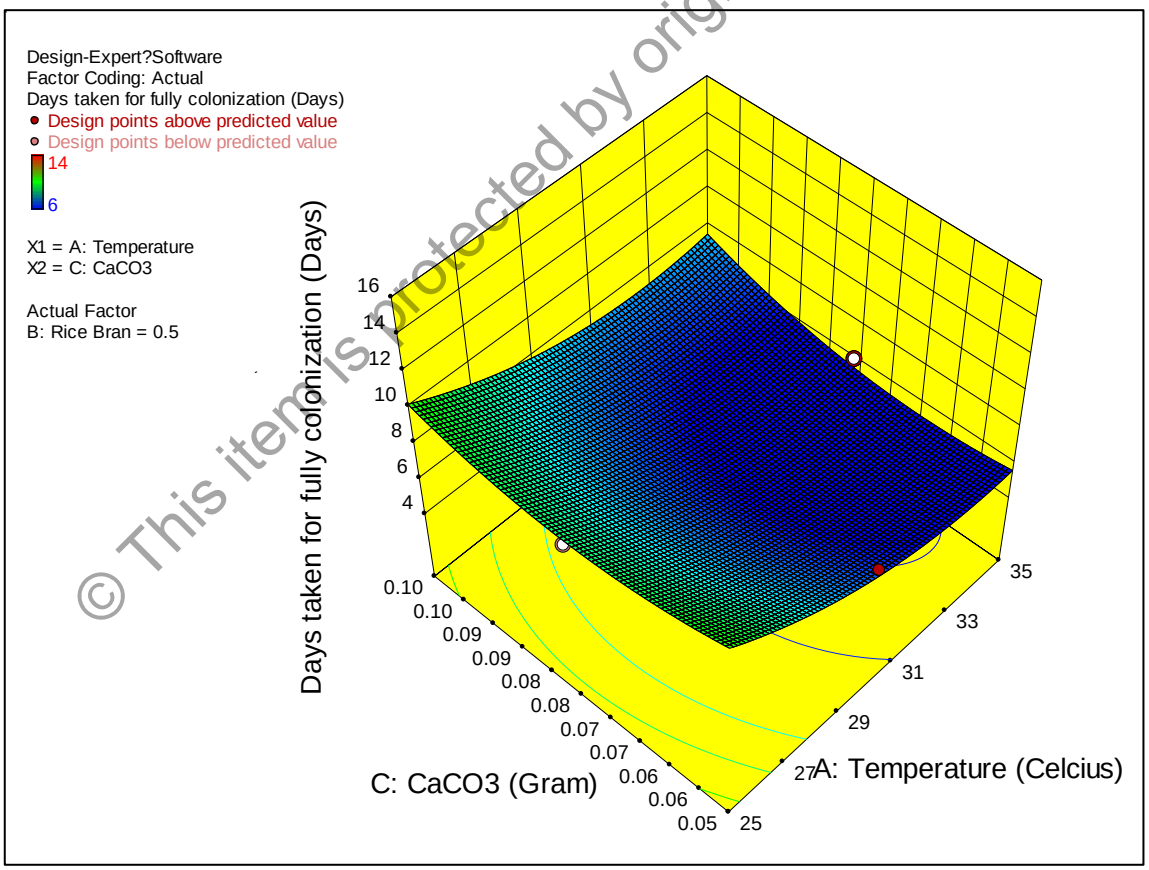

Figure 4.10: Three dimensional response surface plot indicating the interaction between temperature and amount of $CaCO_3$ on days taken for *V. volvacea* mycelium fully colonization on RSD substrate

Figure 4.10 shows the three dimensional response surface plot indicating the interaction between temperature and amount of $CaCO_3$ on days taken for *V. volvacea* mycelium fully colonization on RSD substrate. According to the figure, a similar phenomenon could be seen. When temperature was increased, while $CaCO_3$ was decreased, the minimum days taken for *V. volvacea* mycelium fully colonization on RSD substrate was achieved. Even though, the optimum condition was attained at 35 ˚C and 0.05 gram of $CaCO_3$, as long as the temperature is high enough, no matter the amount of $CaCO_3$ was used, the days taken for *V. volvacea* mycelium fully colonization on RSD substrate could be kept to the minimum.

Based on the research of Ali and his colleagues (Ali *et al.*, 2013), $CaCO_3$ was utilized as to neutralize the acidity in the substrate due to the enzymatic activity occurred to break down the lignocellulosic compounds in RSD. Looking back to the result, it is found out that temperature plays a role in regulating the acidity in the substrate. Thus, the heat provided to the system is sufficient enough to maintain an optimum condition.

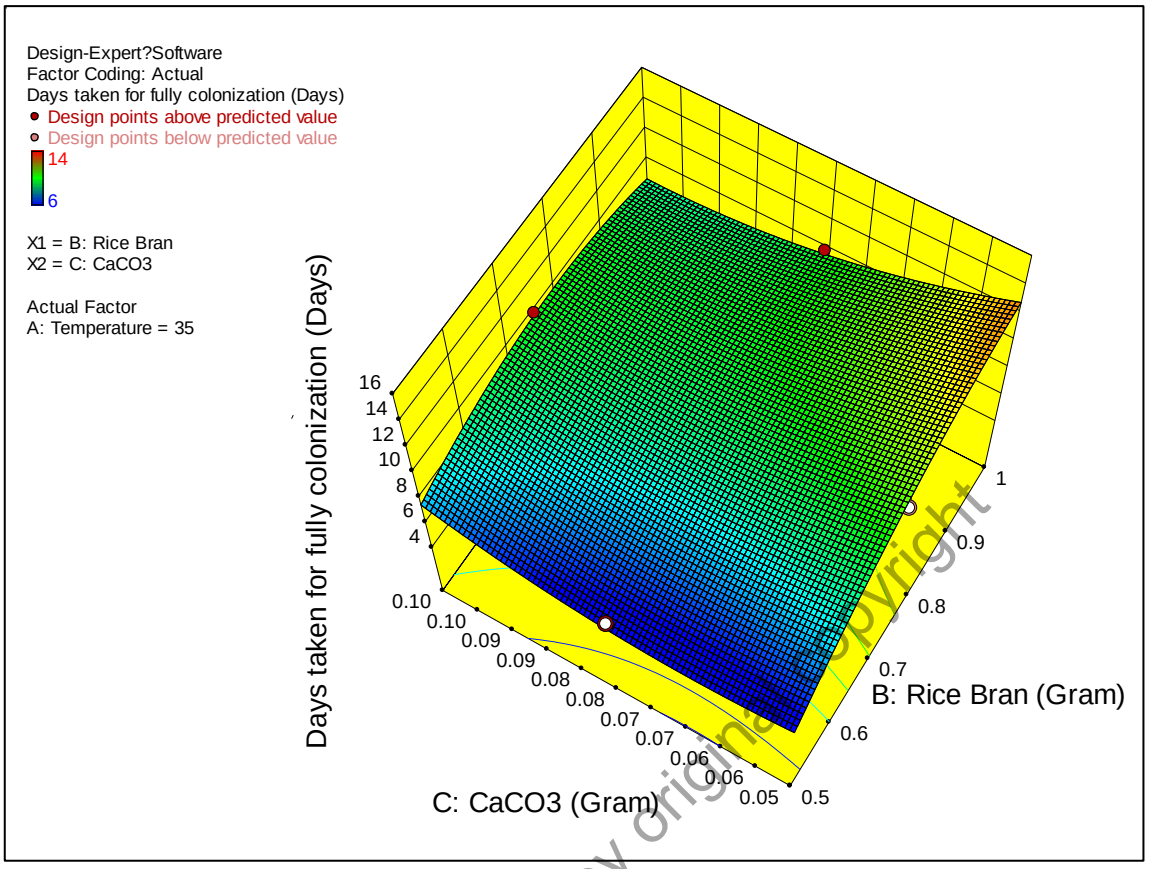

Figure 4.11: Three dimensional response surface plot indicating the interaction between the amount of rice bran and amount of $CaCO_3$ on days taken for *V. volvacea* mycelium fully colonization on RSD substrate

Figure 4.11 demonstrates the three dimensional response surface plot indicating the interaction between the amount of rice bran and amount of $CaCO_3$ on days taken for *V. volvacea* mycelium fully colonization on RSD substrate. Based on the figure, when the amount of $CaCO_3$ increased and the amount of rice bran decreased, the minimum days taken for *V. volvacea* mycelium fully colonization on RSD substrate was accomplished where the optimum condition was reached at 0.08 gram of $CaCO_3$ and 0.50 gram of rice bran.

Aforementioned, both of the additives each serve their own purposes. Judging from the figure, in order to achieve the minimum days taken for *V. volvacea* mycelium fully colonization on RSD substrate, both of the additives must be in adequate amount to achieve so. Based on a few studies published, the combination of rice bran and $CaCO_3$ on RSD was widely used and studied (Marlina *et al.*, 2015; Ali *et al.*, 2013; Ahlawat & Tewari, 2011; Singh *et al.*, 2011; Singh, 2009; Ukoima *et al.*, 2009; Miles & Chang,

2004). Therefore, it has been a must to find out the optimum condition for both additives in order to work perfectly to culture *V. volvacea.*

4.3.3.3 Summary of the Results

The summary of the result from combination of each response surface plots shown that the optimum conditions for minimum days taken for *V. volvacea* mycelium fully colonization on RSD substrate was 34˚C, 0.50 gram of rice bran and 0.07 gram of $CaCO_3$. From the effect and interaction of the days taken for *V. volvacea* mycelium fully colonization on RSD substrate obtained, it could be concluded that *V. volvacea* is really a high temperature mushroom and with it does not need high amount of rice bran to grow. Whereas, adequate amount of $CaCO_3$ was required to control the acidity of the substrate. The result revealed in the ANNOVA is to be expected as technical problem such as exact set point of temperature was not able to obtain as the temperature of the oven is influence by surrounding temperature and inaccuracy of data was not able to achieve.

4.3.4 Validation of Experimental Model

An additional experiment with 30.96˚C, 0.50 gram of rice bran and 0.08 gram of $CaCO_3$ was carried out to verify the validity of the approximation model. The experimental solution given by the validation test was carried out in triplicate and the average days taken for *V. volvacea* mycelium fully colonization on RSD substrate was found to be close to the predicted values given with total percentage error of 1.4287. Thus, this further verify the validation of the model. Table 4.13 shows the average total days taken for *V. volvacea* mycelium fully colonization on RSD substrate of validation solution. The following table shows the desirability ramp for numerical optimisation.

Table 4.16: Average total days taken for *V. volvacea* mycelium fully colonization on RSD substrate of validation solution

	Days taken for *V. volvacea* mycelium fully colonization on RSD substrate
First replicate	5.9000
Second replicate	5.8000
Third replicate	5.7500
Average experimental value	5.8167
Predicted value by statistical model	5.8908
Percentage error (%)	1.4287

Table 4.17: Desirability ramp for numerical optimisation

Name	**Goal**	**Lower Limit**	**Upper Limit**
A: Temperature	Is in range	25.0	35.0
B: Amount of Rice Bran	Is in range	0.5	1.0
C: Amount of $CaCO_3$	Is in range	0.05	0.10
Response 1:Total days taken for *V. volvacea* mycelium fully colonization on RSD substrate	Minimum	6	14

4.4 Determination on the Total Lignocellulosic Content (Extractives, Ash, Lignin, Hemicellulose and Cellulose) of Paddy Straw

Lignin and cellulose was considered as a crucial mechanism to determine the viability of the mushroom. The development of *V. volvacea* was reliant on its ability to degrade the lignocellulosic components present in the substrate on which it is grown (Singh, 2009). Thus, it is vital to learn and understand the total lignocellulosic content of the substrate used which is PS. The total lignocellulosic content which are the extractives content, ash content, lignin content, hemicellulose content and cellulose content in PS was determined by the method shown in subsection 3.6.1, 3.6.2, 3.6.3, 3.6.4 3.6.6 and 3.6.6 respectively.

The reason of carrying out the determination of total lignocellulosic content on PS was to study and understand its lignocellulosic content, structure, composition and characteristics. In order to further understand the lignocellulosic content of the PS, the PS must undergo pretreatment to leach out the extractives step by step. Consequently, special attention was given to the analysis of the extractives, hemicellulose, lignin and cellulose. According to the extractive analysis, a 2:1 ratio of benzene: ethanol solvent is used to leach and extract the non-cell wall part of the PS which consist of alkanes, proteins, monosaccharides and other derivatives.

For hemicellulose and lignin analysis, NaOH was used to extract hemicellulose content while concentrated H_2SO_4 was used to extract lignin. This is due to hemicellulose has shown to have higher solubilisation in alkaline solution rather that acidic solution and alkaline solubilisation of hemicellulose need milder condition such as low alkaline loading, low operating temperature and pressure to operate. While extraction of lignin content require much stronger condition such as high acidic loading, high operating temperature and pressure to break down the structure in order for the hydrolysis to take place (Li *et al.*, 2004). After all the leaching was done, the changes of the weight was measured and recorded down to calculate the lignocellulosic content percentage. Paddy straw was then collected, air dried and shredded using shredder, lastly sieved to a size of less than 150 μm. The purpose of PS sieved to a size of less than 150 μm was to increase the efficiency of leaching where the smaller the surface area of the PS the more lignocellulosic compound can be leached. The compositions were determined and analyzed to evaluate the extractives, hemicellulose, celluloses, lignin and ash which are presented in Table 4.18.

Table 4.18: Total lignocellulosic content of paddy straw

Type of Substrate	Extractives (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Ash (%)
Paddy Straw	11.87±0.505	20.84±6.084	34.12±4.86	10.58±0.052	22.51±1.321

Note: Values are means of 3 replicates.

Judging from Table 4.14, the amount of extractives, hemicellulose, cellulose, lignin and ash is 11.87 %, 20.84 %, 34.12 %, 10.58 %, and 22.51 %. According to some researchers finding on Table 2.5 and Table 2.6, the data obtained is accordance to their research where cellulose was in the range of 32 % to 47 %, hemicellulose was 19 % to 27 %, lignin was 5 % to 24 %, extractives was 8.88 % to15.9 % and ash was 15 % to 24 %. With the composition of PS was greatly compatible to culture straw mushroom and PS was a source of waste that can be found abundant in the environment, therefore PS was chosen as the cultivation medium for straw mushroom.

4.5 Comparison of the Total Days Taken for *V. volvacea* Mycelium to Fully Colonize the Substrate Paddy Straw and Non-composted EFB

According to Table 4.17, it was found out that the total days taken for *V. volvacea* mycelium to fully colonize the substrate paddy straw in "bongkah" was 10 days while for non-composted EFB was 12 days. PS was found out to be faster due to the availability and rich nutrient was higher than non-composted EFB. Paddy straw had a little higher nitrogen, carbon and lower total carbon to nitrogen ratio which were 0.61 %, 54.26 % and 84.0 (Rosmiza *et al.*, 2014). While, non-composted EFB were 0.6 % nitrogen, 44.1 % carbon and 77.7 carbon to nitrogen ratio (Ali *et al.*, 2013). Straw mushroom utilized more carbon source than nitrogen source for the formation of fruiting bodies (Miles & Chang, 2004), therefore the findings was acceptable. The comparison of yield and biological efficiency was not able to proceed due to some technical problem where the process took more time than estimated.

Table 4.19: Total days taken for *V. volvacea* mycelium to fully colonize the "bongkah" subjected to different substrate

Type of substrate	**Days taken for fully colonization (Day)**
PS	10
Non-composted EFB	12