Chapter-5

Effect of Floral Waste Biocompost and Agnihotra ash on Plant Growth

5.1 Material and Method Plant Germination Experiments

To study effect of Agnihotra ash and floral waste biocompost and floral waste bio compost on germination of plant,following water and biocompost were used a. Control soil b. Agnihotra ash and floral waste biocompost with biocompost (1 gm Agnihotra ash and floral waste biocompost + 30 gm biocompost + 70gm soil) The germination process has several stages. The following are some of the steps in seed germination: The seed coat bursts as it absorbs water. It's the first indicator that the seed has germinated. Enzymes are activated, respiration increases, and plant cells are multiplied. A series of chemical changes begins, leading to the formation of the plant embryo. During the germination process, chemical energy held in the form of starch is transformed to sugar. The embryo expands quickly, and the seed coat bursts open. A growing plant emerges from the ground. The tip of the root emerges first, assisting in the anchoring of the seed. It also enables the embryo to absorb nutrients and water from the surrounding environment

Seed Selection

Fig.-18.1 Wheat seeds

Fig.-18.2 Mung seeds

Fig.-18.3 Tomato Seeds

4.2 Statistical analysis by ANOVA: The study was carried out with a completely randomized design with three treatments and 3 replicates, and a pot was the experimental unit for a total of 45 units. All the data were expressed as mean ± standard deviation of three replicates. Statistical test One-way ANOVA (Analysis of Variance) was used, by looking at the effect of soil (T1) vs. soil and biocompost (T3) vs. soil, vermicompost and Agnihotra ash (T3). A one-way ANOVA was used to test the effect of fertilization (soil vs. soil and vermicompost vs. soil, biocompost and Agnihotra ash) on germination rate, shoot length, root length, number of leaves, and plant weight. Statistical analyses were performed using R software (R development Core Team, 2008). If the effect is significant ($p<0.05$), then it will be done with Tukey's advanced test, which aims to see the difference between the different combinations of fertilizers on different parameters.

5.3 Optimization using central composite design (CCD)

A five level two factor rotatable central composite design (α = 1.41) was employed to evaluate influence of independent variables [Agnihotra ash (X_1), Biocompost (X_2)] on quality attributes. The design consisted of four factorial points, four axial points and one centre points with total 9 runs (Table 4) in total. A statistical model incorporating interactive and polynomial terms was used to calculate the responses (K. C. Garala et al., 2013b).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 \quad (1)$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the all trials, andb_i(b_1, b_2, b_{12}, b_{11} and b_{22}) is the estimated coefficient for the corresponding factor X_i (X_1, X_2, X_1X_2, X_{11} andX_{22}), which represents the average result of changing one factor at a time from its low to high value(K. C. Garala & Shah, 2010). The interaction term (X_1X_2) shows how the response changes when two factors are concurrently altered. The polynomial terms (X_1X_1andX_2X_2) are included to investigate the nonlinearity (Patel et al., 2014b).

Trials	**Variable level in coded form**	
	X_1	X_1
1	-1	1
2	1	-1
3	0	+ α
4	0	0
5	-1	-1
6	- α	0
7	0	- α
8	1	1
9	+ α	0
Coded Values	**Actual Values**	
	X_1= Agnihotra ash(gm)	**X_2= Biocompost (gm)**
-1	1	10
0	5.5	55
1	10	100

Table 4. Rotatable central composite design layout

5.4 Result Statistical Analysis of Synergic Effect of Floral Waste Biocompost and Agnihotra Ash on Plant Growth

A one-way ANOVA was used to test the effect of fertilization on five plant growth parameters. There was a significant difference in all the parameters of T1, T2, and T3 (Table 5).

In terms of germination rate over the 23 days, the highest germination rate 94.67% of seed was seen in T3. A one-way ANOVA was used to test the effect of fertilization on plant seed germination. There was a significant difference in the germination rate of plant in T3 effect of compost (Soil, Floral biocompost and Agnihotra ash) on plant weight at the $p<.05$ level for the three conditions [$F(2,6) = 201.2$, $p = 3.17e-06$].

Parameter	**Soil (T_1)**	**Soil & Floral Biocompost (T_2)**	**Soil+ Floral Biocompost + Agnihotra Ash (T_3)**
Germination rate (in %)	71 ±1	85 ±1	94.67 ± 2.08
Shoot Length (in cm)	10.33 ± 0.25	15.37 ± 0.31	17.50 ± 0.26
Root Length (in cm)	9.2± 0.17	12.73± 0.21	13.9± 0.20
No of leaves	6.33± 1.52	8.23±1.68	10.00±1.73
Plant fresh weight in (mg)	380± 0.26	382 ± 0.12	390.6 ± 0.10

Table 5: plant growth parameters of Tomato after 23 Days sowing

Parameter	**DF**		**F value**	**P value**
	Between	**Within**		
Germination rate (in %)	2	6	201.2	3.17e-06
Shoot Length (in cm)	2	6	537.7	1.71e-07
Root Length (in cm)	2	6	475.6	2.46e-07
No of leaves	2	6	268.5	1.35e-06
Plant fresh weight in (mg)	2	6	648.5	9.76e-08

Table 6: plant growth parameters of Tomato after 23 Days sowing

The second growth parameters were increase in shoot length (in Cm) which was observed in T3. There was a significant effect of compost (soil vs. soil and floral biocompost vs. soil, vermicompost and Agnihotra ash) on plant weight at the $p<.05$ level for the three conditions [$F(2,6) = 201.2$, $p = 3.17e-06$].

The root length in the plants over the 23-day period showed the initial highest from treatment T3 (13.9) followed by T2 (12.73), and T1 (9.2) respectively. There was a significant effect of compost (soil vs. soil and floral biocompost vs. soil, vermicompost and Agnihotra ash) on plant weight at the $p<.05$ level for the three conditions [$F(2,6) = 201.2$, $p = 3.17e-06$].

The number of leaves obtained over the 23-day period showed the initial highest from treatment T3 (10.00) followed by T2 (8.23), and T1 (633) respectively. The increase in number of leaves is highly significant based on ANOVA between the three treatments (p= 1.35e-06).

The highest plant weight was observed in T3 (390.6 g) followed by T2 (382 g), and T1 (380 g) respectively. There was a significant difference in the plant weight of plant in T3 effect of compost (Soil, Floral biocompost and Agnihotra ash) on plant weight at the $p<.05$ level for the three conditions [p =9.76e-08].

Post hoc comparisons using the Tukey HSD test indicated that the mixture of soil, biocompost and agnihotra ash have more effect on the plant growth (Supplementary file 2). Specifically, our results suggest that when mixture of all the three ingredient is used, there is significant growth in plant.

5.5 Discussion

The results, as shown in Tables 5, clearly indicates the improvement in plant growth parameters when used the floral biocompost (T2), and agnihotra ash. The mixture of floral biocompost and agnihotra ash added some synergistic effect when used together. The result and statistical analysis clearly suggest that Agnihotra ash and floral waste biocompost assisted germination can therefore be used as a fertilizer. This demonstrates how Agnihotra ash and floral waste biocompost benefit the environment and plant health in a favourable way. Agnihotra's atmosphere and ash can be used as adjuvants in the 'Natural farming' methods also known as the Agnihotra farming methods. Agnihotra Yajna is relatively affordable; it has a positive impact on the environment and soil health. Post hoc comparisons using the Tukey HSD test indicated that the mixture of soil, biocompost and agnihotra ash have more effect on the plant weight. Specifically, our results suggest that when mixture of all the three ingredient is used, there is significant growth in plant. So we concluded that the combined effect of floral waste biocompost and Agnihotra ash has shown positive effects on soil fertility and plant health

5.6 Result of the effect of floral waste biocompost and Agnihotra ash on Plant Growth using Central Composite Design

Germinated mung seed shown in Fig. 18 in which control and experimental both seed’s shoot, root and leaves are observed clearly

Fig. -19 Germinated Mung Seed

Experimental design (CCD)

Preliminary investigations of the process parameters exposed that factors such as Agnihotra ash (X_1) and Biocompost (X_2) exhibited significant influence on germination and overall growth of plant; hence, they were applied for additional systematic studies. All selected dependent variables for all trials showed a wide variation of data (Table 7). The statistics undoubtedly specify the robust impact of X_1 and X_2 on particular responses. The polynomial equations can be used to draw deductions after considering the magnitude of coefficients and the mathematical sign carried: positive or negative (K. Garala et al., 2011).

Trials	X_1 (gm.)	X_2 (gm.)	Y_1 * (˚C)	Y_2* (cm)	Y_3* (cm)
F1	1	100	80.79 ± 1.23	16.21 ± 1.03	12.97 ± 0.81
F2	10	10	62.88 ± 0.93	8.91 ± 1.14	7.55 ± 0.71
F3	5.5	118.45	96.42 ± 1.54	17.85 ± 0.73	15.11 ± 1.25
F4	5.5	55	85.12 ± 2.36	14.98 ± 1.63	12.14 ± 2.14
F5	1	10	71.85 ± 1.35	13.21 ± 2.34	9.94 ± 1.23
F6	0	55	76.21 ± 3.04	11.54 ± 1.54	9.57 ± 1.73
F7	5.5	0	82.09 ± 2.4	10.53 ± 2.48	9.31 ± 1.44
F8	10	100	72.11 ± 1.15	13.28 ± 1.36	10.13 ± 1.87
F9	11.98	55	68.87 ± 2.07	9.53 ± 1.02	8.14 ± 1.19

*All the values are in mean ± S.D. (n=3), X_1=Agnihotra ash (gm.), X_2=Biocompost (gm.), Y_1=Germination (%), Y_2=Shoot Length (cm), Y_3=Root Length (cm).

Table 7 Results of experimental design batches of variables

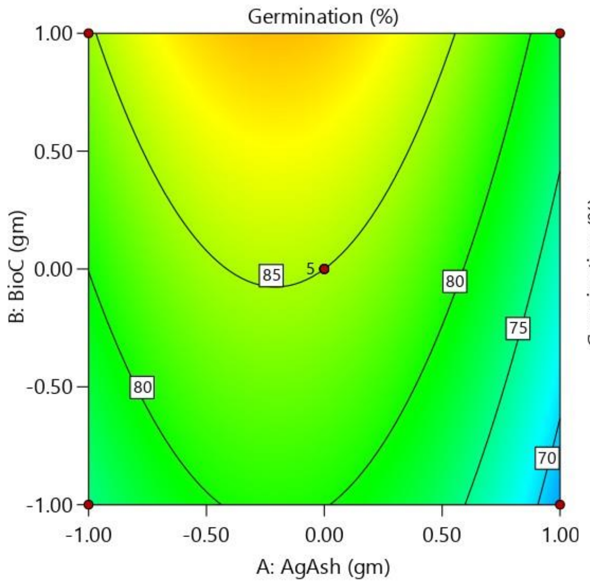
Effect of independent variable on Germination (Y_1)

Concerning Y_1, the results of multiple linear regression analysis showed that the coefficients $b_1$bear a negative sign and b_2 bear a positive sign. The negative of X_1 coefficient indicates that as the amount of Agnihotra ash increases; there is decrease in the germination. But the reduced model implied that it is insignificant at $p \geq 0.05$(Patel et al., 2014a). The positive of X_2 coefficient indicates that as the amount of Biocompost increases; there is increases in the germination. The fitted equation relating the response Y_1 to the transformed factor is shown in following equation,

$$Y_1 = 84.962 - 3.503\ X_1 + 4.804X_2 - 8.454\ X_1^2 - 0.101X_2^2 + 0.072X_1X_2 \quad (2)$$

The Y_1 for all batches F1 to F9 shows good correlation co-efficient of 0.82.Variables which have p value less than 0.05, significantly affect the germination. The relationship between formulation variables (X_1 and X_2) and Y_1 was further elucidated using contour plot and perturbation plot. The effects of X_1 and X_2 on Y_1 are shown in Fig. 20.1 and 20.2. Table 7 showed that with the increase of amount of Agnihotra ash

Up to middle level, i.e., 5.5, germination is increased but after middle level, it was decrease significantly as also observed from perturbation plot (Fig.20.1 and 20.2). It was also found from perturbation plot that upon increasing X_1, germination was also significantly improved.



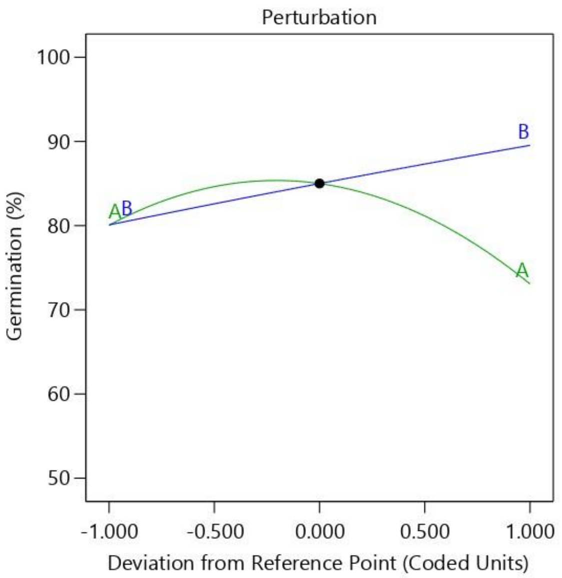


Fig. 20.1 Contour plot showing effect of variables [Agnihotra ash(X_1) and Biocompost (X_2)] on Germination; **Fig. 20.2** the corresponding perturbation plot

Effect of independent variable on Shoot Length (Y_2)

Regarding Y_2, the results of multiple linear regression analysis showed that coefficient b_1 bear negative sign and coefficient b_2 bear a positive sign. The positive X_2 coefficient indicates that as the amount of X_2 (Biocompost) increases; there is increase in the shoot length. Perturbation plot in Figure shows that as amount of X_1 (Agnihotra ash) increase from level -1 to 0, there is slight increase in shoot length and further increase in Agnihotra ash leads to declining the shoot length. The fitted equation relating the response Y_2 to the transformed factor is shown in following equation,

$$Y_2=14.994- 1.259X_1 + 2.215X_2- 2.094X_1^2- 0.267X_2^2+0.343X_1X_2 \quad (3)$$

The Y_2 for all batches F1 to F9 shows good correlation co-efficient of 0.9526. Variables which have p value less than 0.05, significantly affect the shoot length. The relationship between independent variables (X_1 and X_2) and Y_2 was further elucidated using contour plot and perturbation plot. The effects of X_1 and X_2 on Y_2 are shown in Fig. 21.1 and 21.2.
Result of the testing are shown in table. We take different combination and concentration of biocompost and agnihotra ash in which biocompost and agnihotra ash shown positive effect on seed germination and plant growth.

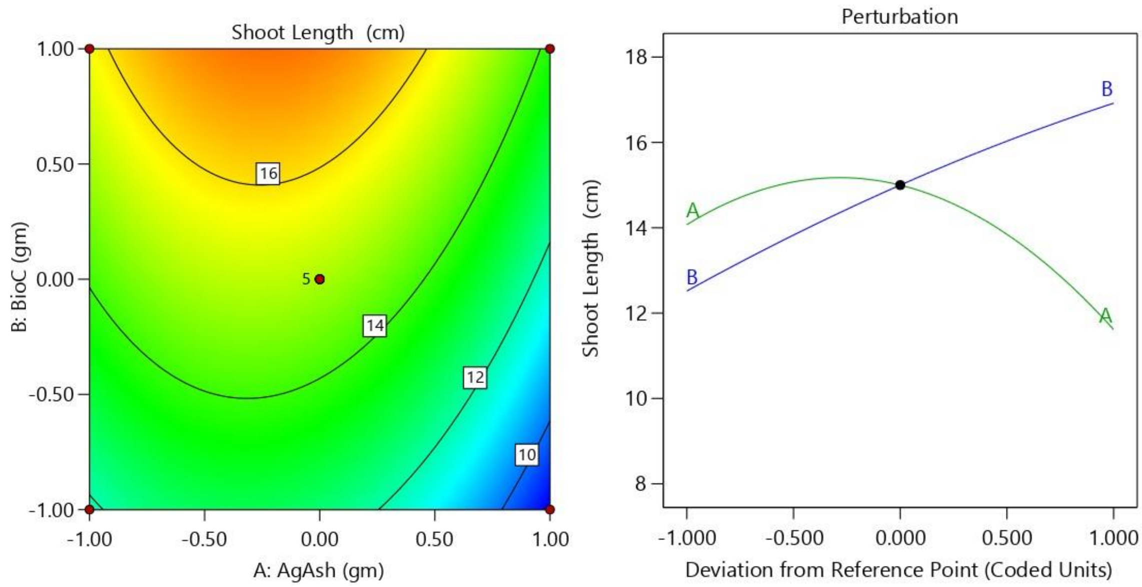


Fig.21.1 Contour plot showing effect of variables [Agnihotra ash(X_1) and Biocompost (X_2)] on Shoot length; **Fig. 21.2** the corresponding perturbation plot

Effect of independent variable on Root Length (Y_3)

About Y_3, the outcomes of multiple linear regression analysis exhibited that the coefficients $b_1$bear a negative sign and b_2 bear a positive sign. The negative of X_1 coefficient indicates that as the amount of X_1 (Agnihotra ash) increases; there is decrease in the root length. Further, as shown perturbation plot (Figure.4), it was found that as we increase X_1 from -1 to 0, there is increase in root length. The positive of X_2 coefficient indicates that as the amount of X_2 (Biocompost) increases; there is increase in the root length, also results confirmed from the perturbation plot (Figure21.1 and 21.2)The fitted equation relating the response Y_3 to the transformed factor is shown in following equation,

$$Y_3 = 12.08 - 0.907X_1 + 1.727X_2 - 1.709X_1^2 - 0.031X_2^2 - 0.113X_1X_2 \quad (4)$$

The Y_3 for all batches F1 to F9 appearances excellent correlation co-efficient of 0.9544. Variables which have p value less than 0.05(Dhingani et al., 2014), significantly affect the root length. The relationship between formulation variables (X_1 and X_2) and Y_3 was further elucidated using contour plot (Figure.22.1 and 22.2).

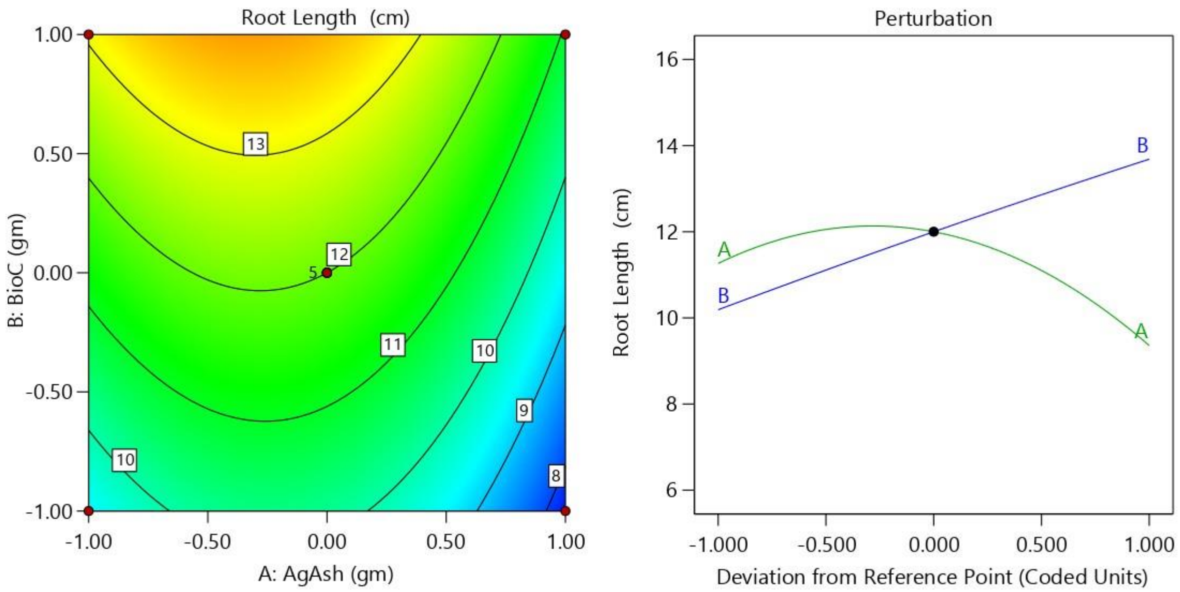


Fig. 22.1 Contour plot showing effect of variables [Agnihotra ash(X_1) and Biocompost (X_2)] on Root length; **Fig. 22.2** the corresponding perturbation plot

Optimization of independent variables

The optimized composition was obtained by applying constraints on dependent variable responses and independent variables. The constraints were maximum germination, shoot length and root length. These constrains were common for all the trials. There commended concentrations of the independent variables were calculated by the Design Expert® version 13

Stat-Ease, Inc., MN,USA)software from the overlay plot (Fig. 23). The optimum values of selected variables obtained were 0.18 (X_1; Agnihotra ash) and 0.69 (X_2; Biocompost. The final optimized composition comprised of 0.81 gm. Agnihotra ash, 31.05 gm. Biocompost. All of the relevant covariates had strong connections to the dependent variables, according to the results of the regression analysis (Raval et al., 2021).

Check point/optimized batch was executed according to the levels of factors optimized. The outcomes depicted non significant ($p > 0.05$) difference and lower magnitude of % relative error between experimentally obtained and the erotically computed data of Germination (%), Shoot Length (cm) and Root Length (cm) as well as significant values of R^2 suggested the robustness of mathematical model and high predictive ability of applied model (K. C. Garala et al., 2013a) (Table 8 to 10).

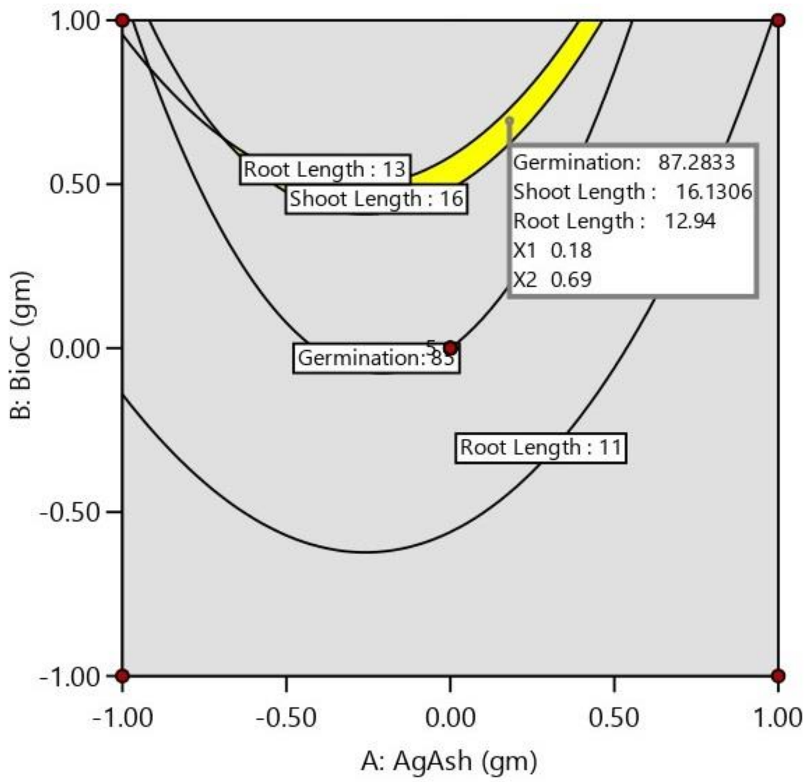


Fig. 23. Overlay plot for optimized parameters

Coefficients	b_0	b_1*	b_2	b_{11}	b_{22}*	b_{12}*
			Germination			
FM	84.962	-3.503	4.804	-8.454	-0.101	0.072
RM	84.891	-	4.804	-8.445	-	-
			Shoot Length			
FM	14.994	-1.259	2.215	-2.094	-0.267	0.343
RM	14.808	-1.259	2.215	-2.060	-	-
			Root Length			
FM	12.080	-0.907	1.727	-1.709	-0.031	-0.113
RM	12.058	-0.907	1.727	-1.705	-	-

FM full model, RM reduced model, ***Response is insignificant at $p \geq 0.05$

Table 8 Summary of regression analysis

		Germination		
	DF	**SS**	**MS**	**R^2**
Regression				
FM	5	787.718	157.543	0.82
RM	2	689.413	344.706	0.72
Error				
FM	7	168.926	24.132	
RM	10	267.230	26.723	
		Shoot Length		
	DF	**SS**	**MS**	**R^2**
Regression				
FM	5	82.928	16.586	0.95
RM	3	81.963	27.321	0.94
Error				
FM	7	4.125	0.589	
RM	9	5.090	0.566	
		Root Length		
	DF	**SS**	**MS**	**R^2**
Regression				
FM	5	51.045	10.209	0.95
RM	3	50.987	16.996	0.95
Error				
FM	7	2.437	0.348	
RM	9	2.495	0.277	

DF, degree of freedom; SS, sum of squares; MS, mean of squares; R^2, regression coefficient.

Table 9 Calculation of testing the model in portions

Response	Predicted value	Experimental value*	%Relative error
Germination (%)	87.28	90.12 ± 2.45	+ 3.15%
Shoot Length (cm)	16.13	15.89 ± 1.03	-1.51%
Root Length (cm)	12.94	13.27 ± 1.17	+ 2.49%

*Values are of mean of three observations ±SD

Table 10 Results of optimized batch

Summary of Work and Conclusion

- **Summary**
- From the suitable sources like weddings, events, worships and temples we collect floral waste for our research work also we found that in Rajkot floral waste generate in large amount.
- We collect cow dung, cow urine, skin, and saliva also gaushala soil sample from Satyakam Gaushala with the help of shepherd
- After collecting the floral waste we dry it and extract liquid material for the media preparation and then prepare floral waste media for isolation of floral waste degrading microbes.
- We inoculate test samples in floral media and after that we isolate and screened floral waste degrading microorganisms. By performing Gram's staining the cultural and morphological characteristics of isolated microorganisms, total seventeen bacterial strains were isolated in which twelve is Gram's Positive and five are Gram's Negative strain.
- Preparation of microbial consortium is next step after isolation, we prepare microbial consortium from the isolated microorganisms and microbial consortium developed successfully and it is very effective it will degrade floral waste within 36 days also we perform trials to check efficiency of microbial consortium at both lab and pilot scale.

- By using developed microbial consortium we produce biocompost from the floral waste in which we found that developed microbial consortia are very effective to degrade floral waste faster than other and produce effective biocompost.

- To check the quality of biocompost we check physical and chemical parameters. Analysis of physic-chemical parameters shows that our finished is better than commercially available compost in which check total 12 different parameters by using suitable procedure and we found our finished biocompost are excellent in quality because amount of N P K and C: N ratio is shows good in finished compost also other parameters like pH, Electrical Conductivity, moisture, Ca, Mg and odour shows good results compare to commercially available compost.

- And in fig. shows the combined effect on seed germination in which positive seen and in table parameter of Growth Parameters of tomato at 23 Days after sowing excellent results compare to control.
- To make more effective biocompost we add agnihotra ash and check the combined effect of biocompost and agnihotra ash on plant growth.
- After checking combined effect we go for statically analysis by ANOVA for better understanding the result and statistical analysis clearly suggest that Agnihotra ash and floral waste biocompost assisted germination can therefore be used as a fertilizer. This demonstrates how Agnihotra ash and floral waste biocompost benefit the environment and plant health in a favorable way.
- Also understanding the combined effect by using Central Composite Design (CCD).The optimum values of selected variables obtained were 0.18 (X_1; Agnihotra ash) and 0.69 (X_2; Biocompost. The final optimized composition comprised of 0.81 gm. Agnihotra ash, 31.05 gm. Biocompost. All of the relevant covariates had strong connections to the dependent variables, according to the results of the regression analysis.

Conclusion

With this research we concluded that microbial flora of Indigenous Gir cow are effective for degradation of floral waste and help to convert floral waste into the biocompost in this study we isolate total seventeen number of bacterial colonies which are capable to degrade floral waste and we developed consortium from this seventeen microbial colonies and after successful enrichment process microbial consortium are able to degrade floral waste speedily it takes around 36 days and after that we make biocompost by using this consortia and finished biocompost is found much better than the commercially available compost. So the present research saws positive results and it helps to reduce environment pollution by degrading floral waste and also helps for production of effective biocompost alternate of chemical fertilizer and which is very useful in organic farming, enhance soil fertility and plant growth also helps to reduce the soil pollution. Agnihotra ash and floral waste biocompost assisted germination and can therefore be used as a fertilizer. This demonstrates how Agnihotra ash and floral waste biocompost benefits the environment and plant health in a favourable way. Agnihotra ash and floral waste biocompost has ensured healthy plant growth. Agnihotra's atmosphere and ash can be used as adjuvants in the 'Natural farming' methods also known as the Agnihotra farming methods. Agnihotra Yajna is relatively affordable; it has a positive impact on the environment and soil health. So we concluded that combined effect of floral waste biocompost and Agnihotra ash shown positive effects on soil fertility and plant health