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# To Find the Efficacy of Rhizobiumspp and Trichodermaspp as Biofertilizer

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## ABSTRACT

*Chemical fertilisers not only affects the target population but show adverse effects on the non-target population and also on the essential organisms of the soil system, hence pose a serious threat to the environment.This paved the way for biofertilizers which improves the soil fertility and the plant growth without causing any damage to the nature. In the present study, Trichodermaharzianum,Rhizobiumsppand non-edible oil seed cake were used as biofertilizers. Efficiency of biofertilizer on the germination paddy by using RSM(Response Surface Methodology) approachwas studied. The Trichodermaharzianum,Rhizobiumspp is an antagonistic fungus which acts as bio-control agent and protects the plant from several soil borne pathogens. It also helps in decomposing the organic matter and helps the plant in the intake of micronutrients*

***Keywords:Trichoderma,RhizobiumsppRSM(Response Surface Methodology), Biofertilizers, paddy, germination.***

**Introduction:** Biological soil fertility management is an ecological approach for sustainable crop production. Different soil micro-organisms play an important role in transformation of nutrients for plant use. Some microorganisms are capable of fixing nitrogen, while some can increase the availability of nitrogen and phosphorus. Biofertilizers are the products containing living cells of micro-organisms that have the ability to mobilize the nutrients from non-usable form through biological processes [1]. Use of Biofertilizers can help to restore the natural fertility of the soil, which is depirved by the use of chemical fertilizers.

Demand for food by the growing human population, has led to an increasing dependence on chemical fertilizers and pesticides. Chemical fertilizers are industrially manipulated substances composed of known quantities of nitrogen, phosphorus and potassium, and their exploitation causes air and ground water pollution by eutrophication of water bodies. The advantages of biofertilizers include longer shelf life causing no adverse effects to ecosystem [3]. They are cost effective, eco-friendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system [2].

## Materials and Methods:

### Chemicals and culture media

Biofertilizer has been prepared by combining *Rhizobium* and *Trichoderma* with non-edible oil seed cake or precomposted oil seed cake. Different combinations of this biofertilizers were added to model plant and their growth was compared in terms of height of the plant, number of tillers with respect to control were studied.

RSM (Response surface methodology) was conducted for paddy germination trials. Different combinations of biofertilizers were added to the soil containing paddy seeds. After an interval of 7days; 10 plants from each combination were collected and their shoot length, root length, shoot weight, root weight was measured and average was taken. The combination which gave maximum root length, shoot length, root weight and shoot weight was selected as best and RSM (Response surface methodology) was carried out for best combination. Selection of levels for optimization was done by OFAT (one factor at a time)for parameter like *Rhizobium,Trichoderma*and precomposted oil seed cake.

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*Trichodermaharzianum*(UASWS 1274; KP114088)was isolated from the root of plant named Dryopteris and cultured in PDB (Potato Dextrose Broth) and molecular identification was carried out at Agarkar Research Institute, Pune.*Rhizobium leguminosarum*(MTCC 9766) was used and sub cultured in YEMA (Yeast Extract Mannitol Agar) Media. Along with *Rhizobiumleguminosarum* and *Trichodermaharzianum*; precomposted oil seed cake is used as a supplement which provides nitrogen and phosphorous to the plant and acts as a good adhesive or carrier of microorganisms. Oil seed cake is precomposted for a month with cow dung in order to remove its toxicity by the action of Enterobacteria present in cow dung.

**PDB media preparation and inoculation of *Trichoderma***

Required volume of PDB (Potato Dextrose Broth) was prepared and autoclaved at 121°C, 15psi pressure for about 15-20 minutes.1% inoculation of *Trichoderma* was maintained in the medium. The culture was then incubated for 7 days at room temperature.

**Biofertilizer Trials on germination of paddy**

The effect of biofertilizers on the growth of different vegetables was observed by adding different combinations of biofertilizers. The different combinations of fertilizers are given below:-

Group 1: Control – only cow dung manure treated

Group 2: only pre-composted non edible oil seed cake

Group 3: pre-composted non edible oil seed cake + *Trichoderma*

Group 4: pre-composted non edible oil seed cake + *Rhizobium*

Group 5: pre-composted non edible oil seed cake + *Trichoderma* + *Rhizobium*

1% of PDB and YEMB media was prepared for the culture of *Trichodermaharzianum* and. Two days and three days incubation at 37°C was provided for *Trichoderma* respectively.

100ml of PDB and 100ml of YEMB media was mixed with 200gm (20%) of non-edible oil seed cake (or precomposted oil seed cake) according to the trials. Mix the above contents with 1Kg of soil and add it to the pots as a top layer, which is initially filled with 9 Kg of soil. The different parameters considered are:-

1. Shoot height
2. Number of tillers

**RSM trials for Paddy germination**

The germination trials were carried for the paddy as per the following combinations.

The different trials are as follows:-

1. Control
2. *Rhizobium* (R)
3. *Trichoderma*(T)
4. *Trichoderma*+ *Rhizobium* (T+R)
5. Non edible oil seed cake (C)
6. Non edible oil seed cake + *Rhizobium* (C+R)
7. Non edible oil seed cake + *Trichoderma* (C+T)
8. Non edible oil seed cake + *Trichoderma* + *Rhizobium* (C+T+R)
9. Pre-composted oil seed cake (PCC)
10. Pre-composted oil seed cake + *Rhizobium* (PCC+R)
11. Pre-composted oil seed cake + *Trichoderma* (PCC+T)
12. Pre-composted oil seed cake + *Rhizobium*+ *Trichoderma* (PCC+R+T)

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**RSM results**

**Optimization of condition for paddy germination trials by Box Behnken design.**

The effect of three parameters i.e. pre-composted cake, *Trichoderma* and *Rhizobium* on paddy germination was examined by Box Behnken Design in levels as shown in Table 4.1.

The variables showing statistically significant effects were tested by STATISTICA software. Factors presenting p-values less than 0.05 were considered to have significant effects on paddy germination. Here a variable is found to be significant, if the size of the effect is less than p=0.05.

Here, independent parameters are,

X1=Pre-composted oil seed cake (g)

X2=*Trichoderma* (ml)

X3=*Rhizobium* (ml)

Dependent parameters are,

Y1=Shoot Length (mm)

Y2=Shoot Weight (mg)

Y3=Root Length (mm)

Y4=Root Weight (mg)

**Table 1,** Box Behnken Design for optimization of independent variables (X1,X2, X3) affecting paddy germination trials with observed dependent response (Y1, Y2, Y3, Y4).

| Run No. | X1 | X2 | X3 | Y1 | Y2 | Y3 | Y4 |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 200 | 21.67 | 77.1 | 7.82 | 18 |
| 2 | 40 | 0 | 200 | 3.575 | 9.25 | 0.2 | 5.5 |
| 3 | 0 | 40 | 200 | 20.28 | 65.2 | 6.14 | 21.7 |
| 4 | 40 | 40 | 200 | 4.98 | 19 | 0.675 | 13.8 |
| 5 | 0 | 20 | 120 | 20.24 | 80.1 | 6.79 | 53.6 |
| 6 | 40 | 20 | 120 | 2.75 | 19 | 0 | 0 |
| 7 | 0 | 20 | 280 | 21.46 | 85.7 | 6.06 | 39.1 |
| 8 | 40 | 20 | 280 | 3.528 | 12.143 | 0.228 | 7.143 |
| 9 | 20 | 0 | 120 | 16.85 | 85.2 | 3.02 | 16.8 |
| 10 | 20 | 40 | 120 | 17.85 | 69.9 | 4.08 | 17.6 |
| 11 | 20 | 0 | 280 | 20.14 | 96.4 | 6.27 | 19.8 |
| 12 | 20 | 40 | 280 | 18.67 | 92.9 | 3.99 | 35 |
| 13 | 20 | 20 | 200 | 17.5 | 81.1 | 5.12 | 35.4 |
| 14 | 20 | 20 | 200 | 17.09 | 80.6 | 4.93 | 36.7 |
| 15 | 20 | 20 | 200 | 17.21 | 79.5 | 4.65 | 37.4 |

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**Table2:**Table for shoot length.

| | SS | df | MS | F | p |
|---|---|---|---|---|---|
| **Independent variables** | | | | | |
| **X1 (L)** | **592.66** | **1** | **592.66** | **2544.24** | **5.78E-08** |
| **X1 (Q)** | **112.37** | **1** | **112.37** | **482.40** | **3.63E-06** |
| X2 (L) | 0.03 | 1 | 0.03 | 0.13 | 0.731 |
| **X2 (Q)** | **2.77** | **1** | **2.77** | **11.89** | **0.018** |
| **X3 (L)** | **4.66** | **1** | **4.66** | **20.01** | **0.006** |
| X3 (Q) | 0.22 | 1 | 0.22 | 0.94 | 0.374 |
| **Interactions** | | | | | |
| X1 *X2 | 1.89 | 1 | 1.89 | 8.14 | 0.035 |
| X1 * X3 | 0.04 | 1 | 0.04 | 0.20 | 0.666 |
| X2 * X3 | 1.52 | 1 | 1.52 | 6.54 | 0.050 |
| Error | 1.16 | 5 | 0.23 | | |
| **Total SS** | **721.79** | **14** | | **$R^2$** | **0.9983** |

The response surface graphs (RSG) for pre-composted oil seed cake, *Trichoderma* and *Rhizobium* as a function of Shoot length were determined.

The optimization of variables (X1, X2, X3) were determined using desirability profiles for Y1 (Shoot length). The regression analysis obtained after ANOVA resulted in the following second order equation for the response as a function of independent variables (X1, X2 and X3).

**$Y1=19.7+10.06*X1-137.9*X1^2-4.69*X2+21.65*X2^2+0.336*X3+0.382*X3^2+17.21*X1X2-0.691*X1X3-3.859*X2X3$**

The $R^2$ values for the model predicting the main response i.e. shoot length was found to be 0.998, indicating that the model is significant.

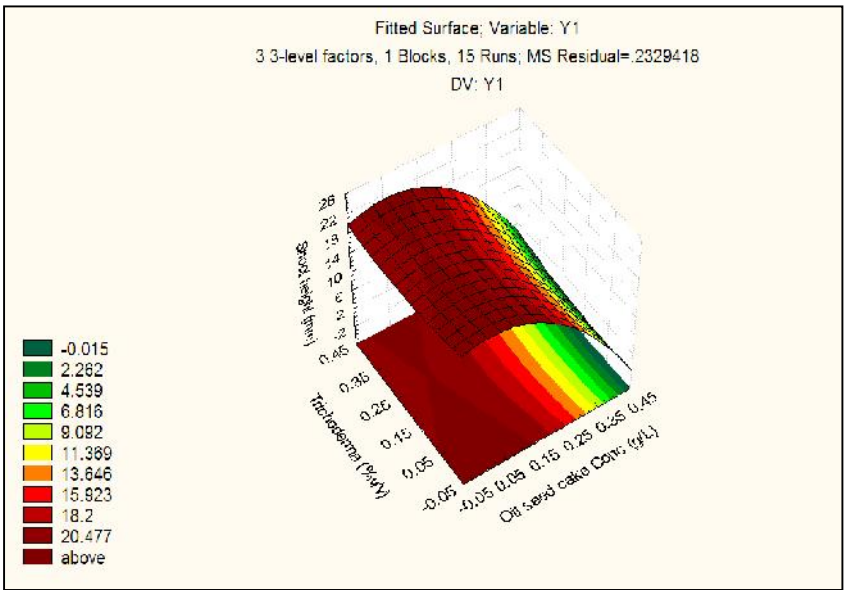


**Fig. 1:** Response surface plots showing the effect of Pre-composted oil seed cake concentration and *Trichoderma* concentration on growth of paddy shoot length.

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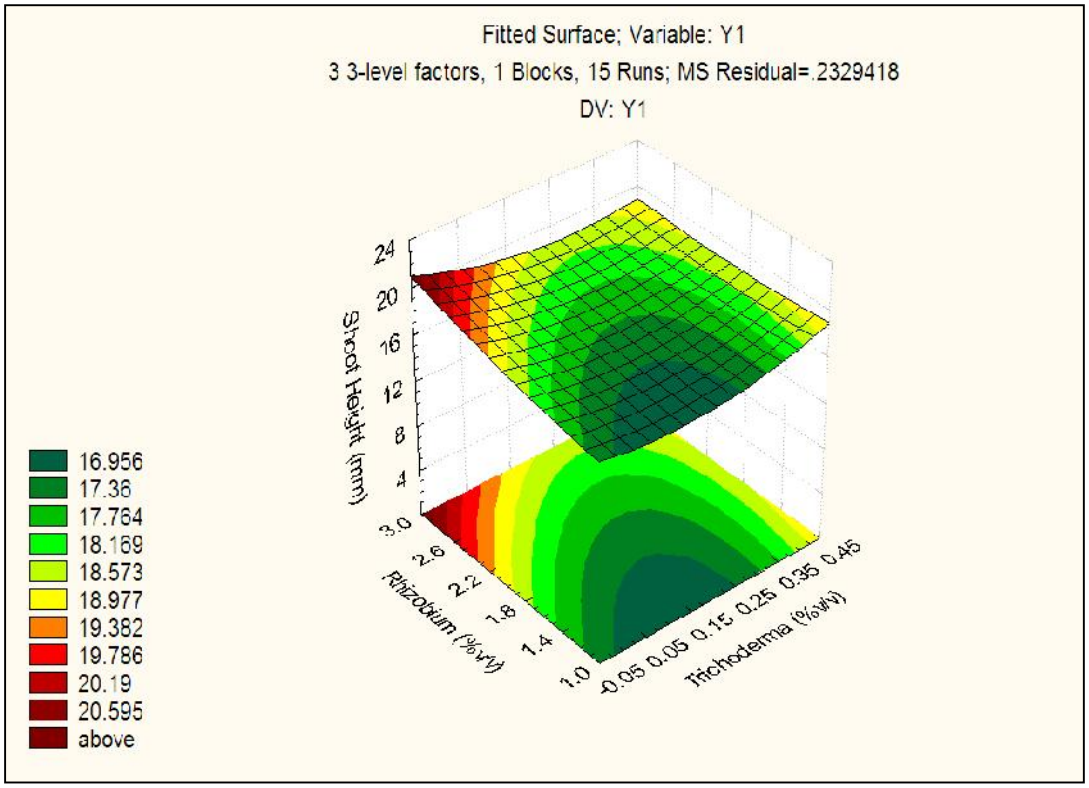


**Fig. 2:** Response surface plots showing the effect of *Rhizobium* concentration and *Trichoderma* concentration on growth of paddy shoot length.

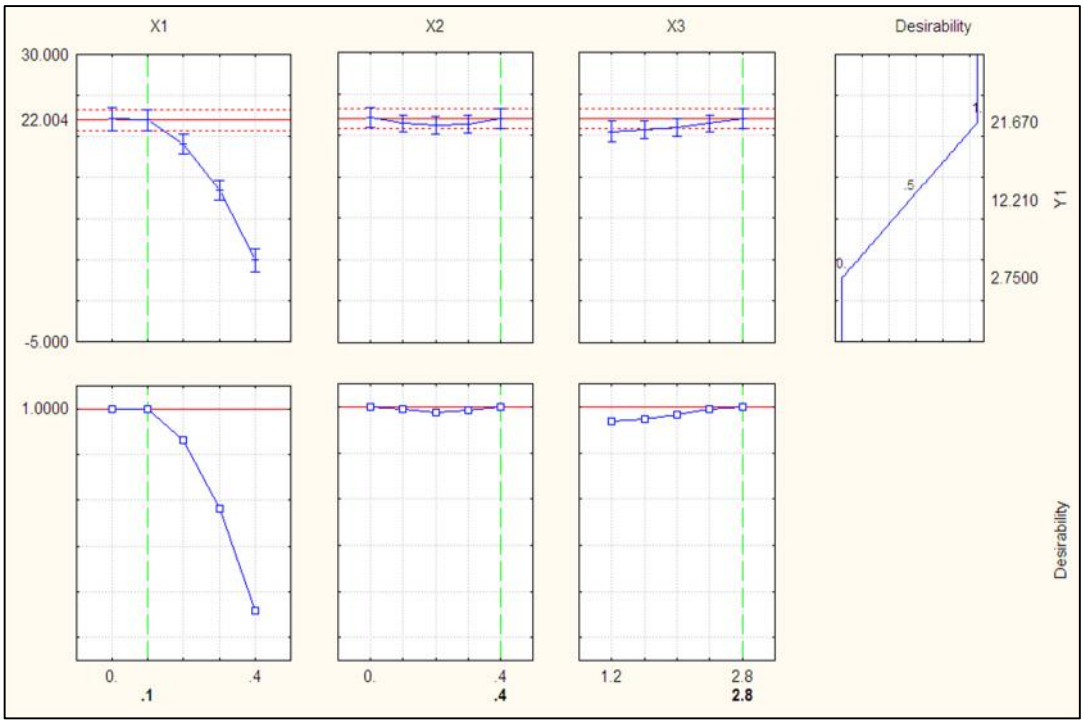


**Fig. 3:** Profiles for predicted shoot length and the desirability levels for different parameters for optimum paddy growth.

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**Table 3:** Table for shoot weight.

| | SS | df | MS | F | p |
|---|---|---|---|---|---|
| **Independent variables** | | | | | |
| **X1 (L)** | **7731.89** | **1** | **7731.89** | **157.01** | **5.75E-05** |
| **X1 (Q)** | **5140.75** | **1** | **5140.75** | **104.39** | **0.0001** |
| X2 (L) | 54.86 | 1 | 54.86 | 1.11 | 0.3395 |
| X2 (Q) | 0.74 | 1 | 0.74 | 0.01 | 0.9069 |
| X3 (L) | 135.65 | 1 | 135.65 | 2.75 | 0.1578 |
| X3 (Q) | 139.61 | 1 | 139.61 | 2.83 | 0.1530 |
| **Interactions** | | | | | |
| X1*X2 | 117.18 | 1 | 117.18 | 2.37 | 0.1835 |
| X1*X3 | 38.79 | 1 | 38.79 | 0.78 | 0.4154 |
| X2*X3 | 34.81 | 1 | 34.81 | 0.70 | 0.4388 |
| Error | 246.22 | 5 | 49.24 | | |
| **Total SS** | **13814.12** | **14** | | **$R^2$** | **0.9821** |

The response surface graphs (RSG) for pre-composted oil seed cake, *Trichoderma* and *Rhizobium* as a function of Shoot weight were determined.

The optimization of variables (X1, X2, X3) were determined using desirability profiles for Y2 (Shoot weight). The regression analysis obtained after ANOVA resulted in the following second order equation for the response as a function of independent variables (X1, X2 and X3).

**$Y2=109.48+229.5*X1-932.3*X1^2-74.54*X2-11.22*X2^2-33.07*X3+9.60*X3^2+135.3*X1X2-19.46*X1X3-+18.438X2X3$**

The $R^2$ values for the model predicting the main response i.e. shoot weight was found to be 0.982, indicating that the model is significant.

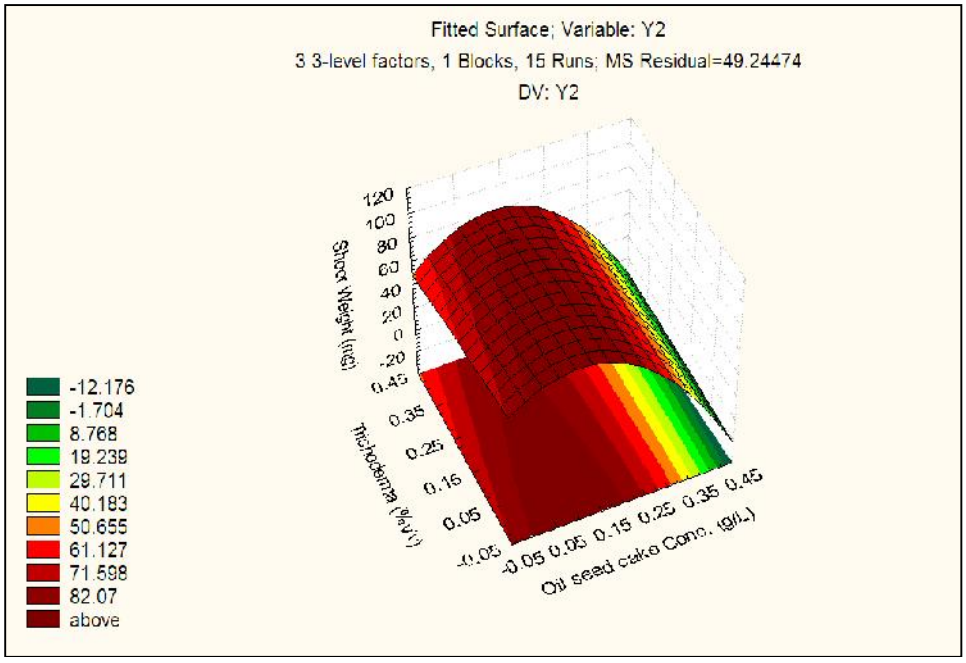


**Fig. 4:** Response surface plots showing the effect pre-composted oil seed cake concentration and *Trichoderma* concentration on growth of paddy shoot weight.

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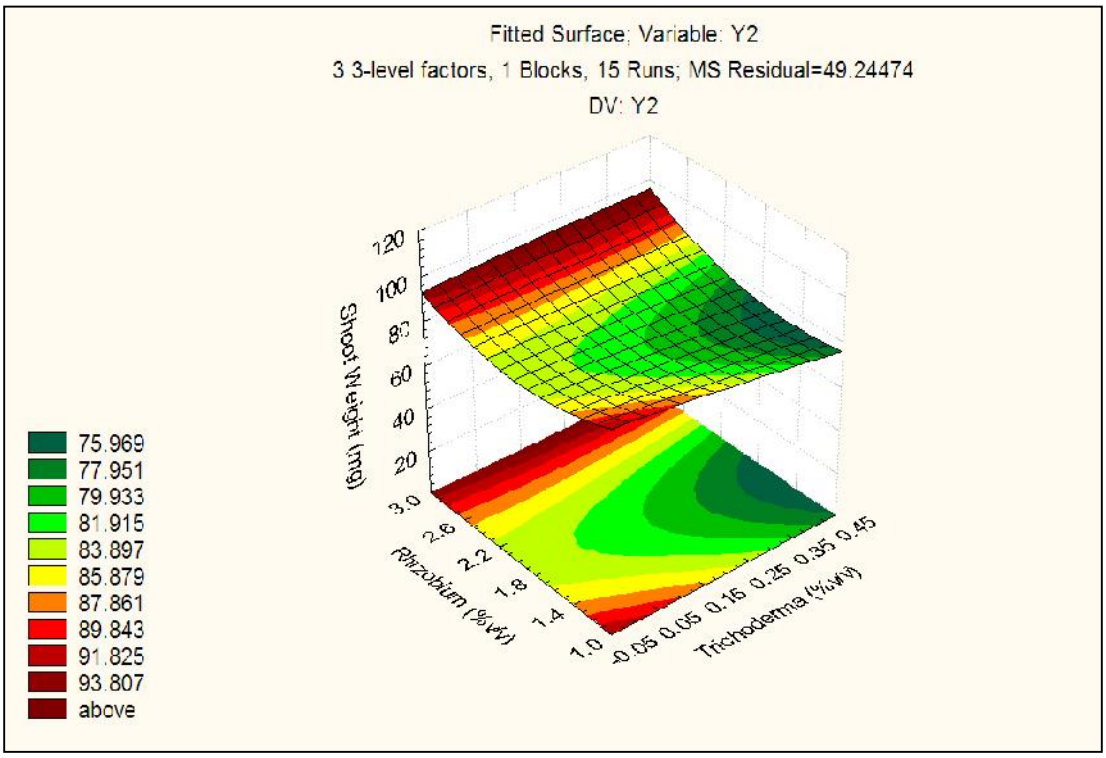


**Fig.5:** Response surface plots showing the effect *Trichoderma* concentration and *Rhizobium* concentration on growth of paddy shoot weight.

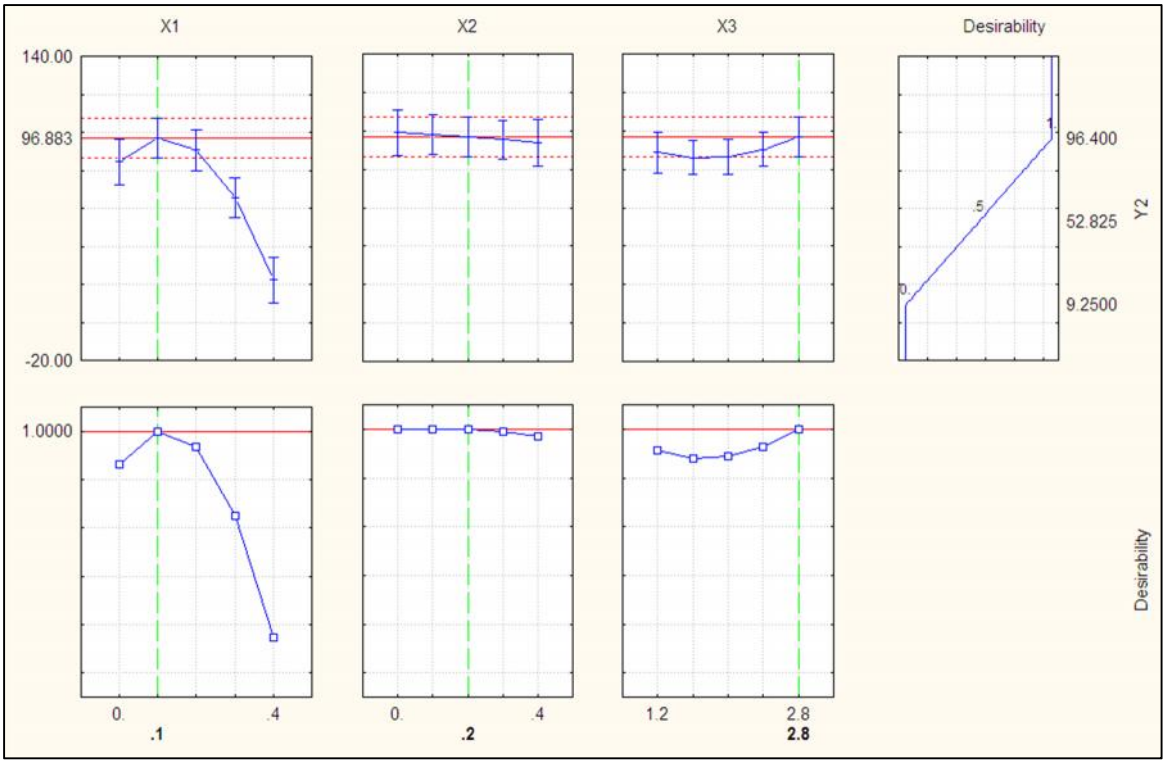


**Fig.6:** Profiles for predicted shoot weight and the desirability levels for different parameters for optimum paddy growth.

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**Table 5:** Table for root length.

| | SS | df | MS | F | p |
|---|---|---|---|---|---|
| **Independent variables** | | | | | |
| **X1 (L)** | **82.60** | **1** | **82.60** | **227.57** | **2.32E-05** |
| **X1 (Q)** | **4.72** | **1** | **4.72** | **13.01** | **0.01** |
| X2 (L) | 0.73 | 1 | 0.73 | 2.02 | 0.21 |
| X2 (Q) | 0.01 | 1 | 0.01 | 0.03 | 0.85 |
| X3 (L) | 0.88 | 1 | 0.88 | 2.43 | 0.17 |
| X3 (Q) | 0.92 | 1 | 0.92 | 2.53 | 0.17 |
| **Interactions** | | | | | |
| X1*X2 | 1.16 | 1 | 1.16 | 3.19 | 0.13 |
| X1*X3 | 0.22 | 1 | 0.22 | 0.63 | 0.46 |
| **X2*X3** | **2.78** | **1** | **2.78** | **7.68** | **0.03** |
| Error | 1.81 | 5 | 0.36 | | |
| **Total SS** | **95.58** | **14** | | **$R^2$** | **0.9810** |

The response surface graphs (RSG) for pre-composted oil seed cake, *Trichoderma* and *Rhizobium* as a function of root length were determined.

The optimization of variables (X1, X2, X3) were determined using desirability profiles for Y3 (Root length). The regression analysis obtained after ANOVA resulted in the following second order equation for the response as a function of independent variables (X1, X2 and X3).

$$\mathbf{Y3=2.32-10.44*X1-28.27*X1^2+6.83*X2-1.5*X2^2+4.28*X3-0.78*X3^2+13.46*X1X2+1.49*X1X3-5.21X2X3}$$

Media preparation for the microbial growth followed the same procedure as for the model plants. 10ml of PDB and 10ml of YEMB media was mixed with 20gm (20%) of non-edible oil seed cake (or precomposted oil seed cake) as per the respective trials. Mix the above contents with 100g of soil and add it to the bags initially filled with about 900g of soil as a top layer. Seeds of paddy were sowed in to those bags containing soil.

In the above 12 trials, trial showing best result in terms of germination and further growth of the paddy for a period of 7 days was subjected to optimization using RSM (Response Surface Methodology).This study used the microorganisms like *Trichodermaharzianum* along with the non-edible oil seed cake as biofertilizer to enhance the crop yield. *Trichodermaharzianum*is one of the best biocontrol agents effective against various soil borne pathogens. Itsecretes lytic enzymes and antibiotics to lyse the soil pathogens and hence protects the plant. It also secretes plant growth promoting hormones and helps the plants in their growth. The decomposition of organic matter is enhanced along with the increase in phosphorous utilisation by the plant.

Initially paddy germination trials were carried where non edible oil seed cake was used without precomposting and hence there was no germination of paddy. Non edible oil seed cake is found to contain the toxins which are too strong for the paddy germination. Hence the pre composted oil seed cake was used for the next trials where cow dung is mixed in equal quantity with cake and kept for about a month. Enterobacteria present in the cowdung are known to reduce the toxicity of the oil cake which acts as a rich source of nutrition for the plant. For the paddy germination trials, OFAT (One Factor At a Time) analysis was carried out for three parameters *Trichodermaharzianum (ml),* Non-edible oil seed cake (g)) taken. The responses measured were shootlength, shoot weight, root weight and root length.The optimum values found were 200 ml, 20 ml and 20 g for, *Trichodermaharzianum* and non-edible oil seed cake respectively. The RSM trials werecarried for these OFAT values to get the most accurate optimum values.

**Conclusion:**

This study used the microorganisms *Trichodermaharzianum* along with the non-edible oil seed cake as biofertilizer to enhance the crop yield. *Trichodermaharzianum*is one of the best biocontrol agents effective against various soil borne pathogens.Itsecretes lytic enzymes and antibiotics to lyse the soil pathogens and hence protects the plant. It also secretes plant growth promoting hormones and helps the plants in their growth. The decomposition of organic matter is enhanced along with the increase in phosphorous utilisation by the plant.

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