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Formulation of potential vegetable waste compost in association with microorganisms and *Spirulina platensis*

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ABSTRACT

*Comparative study was undertaken to evaluate efficiency of the soil organisms like -Rhizobium, Azotobacter & Lactobacillus and Spirulina in different combinations along with vegetable waste to formulate it into a biofertilizer and in enhancing the growth of green gram plants. The study consists of experimental groups, control and a test control with different combinations. Group 1 contains the combination of the three bacteria along with a cyanobacterial member i.e. Spirulina and (**R+A+L**), the group 2 contains the **R+A+L,** group3 contains **R+L** and the group 4 contains **A+L**. Group5 contains a set- up as test control and a control was set-up. The Rhizobium and Azotobacter are the nitrogen fixing organisms, where as Lactobacillus produce lactic acid from sugars and other carbohydrates. Apart from this, Lactic acid is a strong sterilizing compound and suppresses harmful microorganisms and enhances decomposition of organic matter, thereby removing undesirable effects of undecomposed organic matter. The cyanobacterial member i.e. Spirulina is a rich protein as well as nitrogen provider for the plants The ratio of Vegetable waste and soil has taken in 1:3 ratio.1 ml broth cultures (10^9 in numbers and 25gms of Spirulina) of each organism inoculated into the vegetable waste + soil mixture. The study results reveals that group 1 has shown a greater impact on the growth of the plant in terms of germination percent growth of the plants in terms of shoot length and root length, cholorphyll and protein of the leaf was high when compared with other experimental test control and control groups. So, the vegetable waste along with efficient soil organisms is potential in promoting growth of crops.*

Key words: *Rhizobium*; *Azotobacter*; *Lactobacillus*; *Spirulina;* Vegetable waste; Biofertilizer; Green gram plants.

INTRODUCTION

India is second largest populous country in the world. The increasing population has decreased the cultivable land resources day by day, to meet the food, fiber, fuel, fodder and other needs of the growing population. The productivity of agricultural land and soil quality needs improvement in zinc, iron copper [1, 2] and potassium [3]. The post independence era has started green revolution which has shown a path to developing countries for self -sufficiency in food [4].

Green revolution encouraged synthetic agro-chemicals like fertilizers and pesticides, high-yielding varieties of crops and exploration of material irrigation potentials etc. However, continuous use of these high energy inputs led to decline in production and deterioration of soil health by erosion and pollution.

The extensive usage of chemicals fertilizers and pesticides caused number of deaths and illness to the farmers. The poor farm management technique and improper use of agro-chemicals has also resulted in both soil quality and environmental degradation [5]. The use of non-chemical fertilizers and pesticides is one of the common practices that have been introduced with alternative agricultural systems, which includes the use of biofertilizer [6]. Biofertilizer are commonly referred to the fertilizer that contains living microorganisms that their activities will promote soil ecosystem and produce supplementary substance for the plants [7].

The microorganisms and the nutrients present in the raw materials are very helpful in improving soil health. There are different types of biofertilizers available that their differences are mainly the raw materials used, forms of utilization and the sources of microorganisms [8].

In nature the organic matter and debris is disposed off as compost which maintains the soil fertility. During the last two decades there has been a significant sensitization of global community towards health and in maintaining soil fertility, crop productivity and food quality. This can be achieved by organic farming which prevents the degradations of fertile layers of soil and makes the soil potential to produce highyield, nutritious and healthy food. Pests can also be managed by using compost based fertilizers which are potential biological control measures.

Vegetable waste provides good amount of nutrients for inhabiting microbes, they are neither pathogens nor concerned with human health. However they are prone to strong odors during decomposition. The high moisture contain of vegetable waste causes expensive to dispose it off. The vegetable waste containing lettuce and onion waste has high amounts of sulphur 0.2% and 0.7% respectively; their moisture content is of 96.2% and 91.1% respectively [9]. The breaking down of organic material of compost is aerobic and performed by bacteria, fungi, insects and animals which inhabit the soil. These organisms utilize the complex nutrients of the compost and release the essential minerals and elements into soil which in turn available easily for crops and enhance their yield and provide healthy and nutritious food to the mankind. The present study was undertaken to dispose off the waste generated domestically for a useful practice and by converting the waste into compost. The utilization of chemical fertilizer gradually decrease which ultimately increases the soil fertility which in turn also reflects on the improvement of nutritive values of food and also improvement of health of mankind.

MATERIALS AND METHODS

2.1 Preparation of vegetable waste compost

House hold vegetable peelings which thrown as waste were collected, sun-dried and powdered. Now the vegetable waste powder is mixed with soil in 1:3 proportion and these mixtures are watered and mixed now and then to get homogeneous compost. The compost mix was allowed to settle for week.

2.2 Inoculation of selective organisms into compost
The settled soil and vegetable waste powder mix was sterilized by the process of tyndalization consequently for three days. After sterilization soil organisms of 1ml broth cultures of Rhizobium, *Azotobacter*, *lactobacillus* (10^9 organisms) and *Spirulina* (25 grams) [10] were inoculated into soil waste mix. The inoculation was done in different combinations which are as follows

- *R+A+L+S* *(RHIZOBIUM+AZOTOBACTER+LACTOBACILLUS+SPIRULINA)*
- *R+A+L* *(RHIZOBIUM+AZOTOBACTER+LACTOBACILLUS)*
- *R+L* *(RHIZOBIUM+LACTOBACILLUS)*
- *A+L* *(AZOTOBACTER+LACTOBACILLUS)*
- TEST CONTROL (WITH CHEMICAL FERTILIZER AND WITHOUT INOCULATION)
- CONTROL (WITHOUT INOCULATION)

The inoculated mixture was incubated for 3weeks.

2.3 Productions of crops using vegetable compost
The green gram seeds (*Cicer arietinum*) soaked overnight in sterile distilled water were sown in pots containing soil+vegetable compost inoculated with different combinations of organisms as said above. In each set 30 seeds were sown. The seedlings were germinated within 48 to 72hrs and growth of plantlets was observed for 45days and various parameters were analyzed and results were recorded and shown in table 2 & 3.

2.4 Estimation of chlorophyll
The chlorophyll content in green gram leaves was estimated by the method [11, 12] shown in table 4.

Procedure
Chlorophyll was extracted from 1g of the sample using 20ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5 minutes. The extraction was repeated until the residue became colorless. The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer at 645 and 663nm against 80% acetone blank. The amount of total chlorophyll in the sample was calculated using the formula,

$$\text{Total chlorophyll} = 20.2\,(A645) + 8.02\,(A663) \times V/1000 \times W$$

where,
V = final volume of the extract
W = fresh weight of the leaves
The values are expressed as mg chlorophyll/g sample.
The analysis in the leaves of *green gram* plant revealed that the leaves were the richest source of chlorophyll.

2.5 Estimation of protein

After 45days of plant growth the product (seeds) collected. Protein estimation of the product as done. The estimated protein calculated by comparing the O.D values obtained with standard BSA O.D values. By plotting the standard with obtained values the concentration of protein was measured. Thus the protein of product was estimated [13] and shown in table 5.

Procedure

The sample preparation from each assay was used as the protein source for estimation. Standards corresponding to 0.2 to 1.0ml (20-100µg) were pipetted out into a series of test tubes and 10µl of the sample extract was used for the estimation. The volume was made up to 1.0ml in all the tubes with distilled water. To this, 5.0ml of solution 3 was added, mixed well and incubated at 37°C for 3 minutes. Then 0.5ml of Folin-Ciocalteau reagent was added, mixed well and incubated at 37°C for 3 minutes. The blue colour developed was read at 660nm in a spectrophotometer.

RESULTS AND DISCUSSION

The vegetable waste compost and soil mixture after incubation with cultures was tested for the presence of organic elements and also the nature of the soil was examined at a government organization-The Soil Fertility Testing Centre, Anakapalle, Visakhapatnam, A.P. India. The results are as presented in table. The percent of germination rate was tabulated in table 2.The average lengths of root and shoot calculated and mentioned in table 3. The amount of Chlorophyll estimated has been shown in table 4.Protein estimation results tabulated in table 5.

The vegetable waste is highly organic, rich in carbohydrates and proteins. The organisms Rhizobium, *Azotobacter* and *Lactobacillus* have utilized these complex organic substances and converted the waste into a reusable fertilizer by improving the quality of compost with adequate amounts of organic carbon and nitrogen [14]. By application of this fertilizer the seeds of green gram has shown better rate of germination and growth. The soil characters which have been tested are shown clear cut evidence of variation for soil nature, p^H, salt concentration, organic carbon, phosphorous, potassium and nitrogen for different cultures inoculated into the vegetable waste [15,16,17]. The values of shoot length and root lengths of different setups show the clear differentiation and the highest values observed in case of R+A+L+S. The very nearer values have been shown by R+A+L. R+L and A+L have shown comparatively lower values than the other two but, higher or similar with the test control / control. The values of chlorophyll estimation and protein are also higher in case of R+A+L+S. Because of presence of spirulina which is a potential source of chlorophyll and nitrogen and other bacteria which can fix atmospheric nitrogen [18]. The increase of chlorophyll and protein in the product was observed. The increase in chlorophyll shows good growth of plants. R+A+L has shown less differentiation with R+A+L+S and variation with all other experimental setups. The R+L and A+L have clearly shown differences in all parameters with the above two . But slightly higher are equal to Test control/control. By over all study of these results it is clearly proved that the protein and chlorophyll content of green gram seeds has been enhanced by the application of vegetable waste compost with different cultures. The test control with chemical fertilizer has shown slightly higher or nearer values with control and are less values with microbial cultures.

Table 1. Soil +vegetable waste compost analysis report

S.no	CHARACTERS	R+A+L+S	R+A+L	R+L	A+L	CONTROL	TEST CONTROL
1	Soil nature	S-I	S-I	S-I	S-I	S-I	S-I
2	P^H	6.90	6.96	7.04	7.14	7.40	7.40
3	Salt conc.(mM/cm)	0.72	0.56	0.36	0.32	0.21	0.21
4	Organic carbon	H	H	M	H	L	M
5	Phosphorous(Kg/Hectare)	33	31	42	36	28	45
6	Potassium(Kg/Hectare)	259	259	259	259	116	259
7	Nitrogen(Kg/Hectare)	H	H	M	M	L	M

S-1 - Type 1 soil ; H- High; M - Medium,; L - Low.

Table 2. Germination rate of plants

S.no	Groups*	% of germination rate
1.	R+A+L+S	99%
2.	R+A+L	95%
3.	R+L	80%
4.	A+L	90%
5.	Control	80%
6.	TEST control	85%

Table 3. Mean shoot and root lengths of the seedlings with different groups

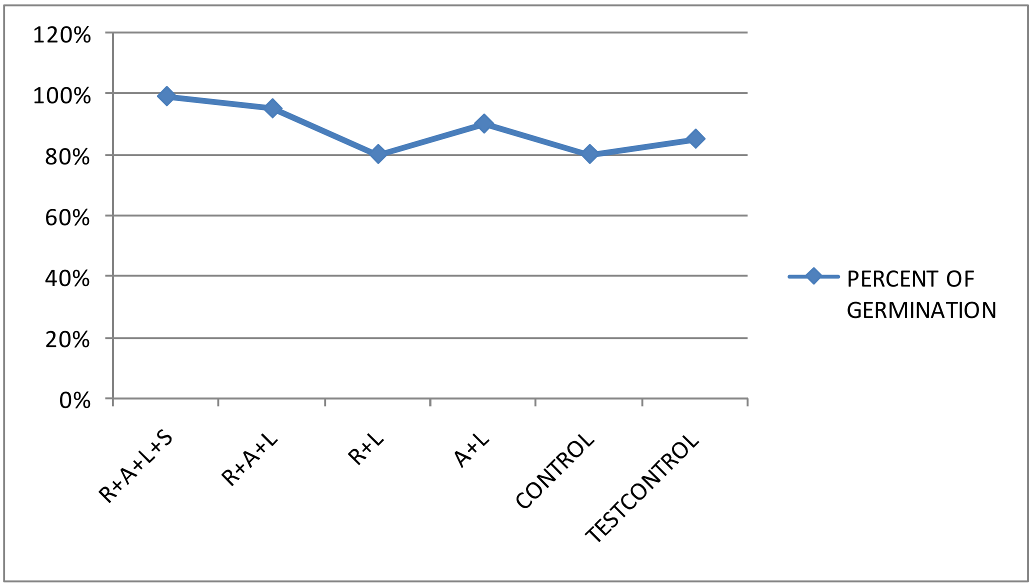
S.no	Groups	Mean shoot length in cms	Mean root Length in cms	Total length of plantlets in cms
1	R+A+L+S	23.3	9.0	32.3
2	R+A+L	25.3	5.9	31.2
3	R+L	24.6	5.2	29.8
4	A+L	22.0	3.9	25.9
5	Control	16.0	5.0	21.0
6	Test control	20.0	5.0	25.0

Table 4. Estimation of chlorphyll from leaves

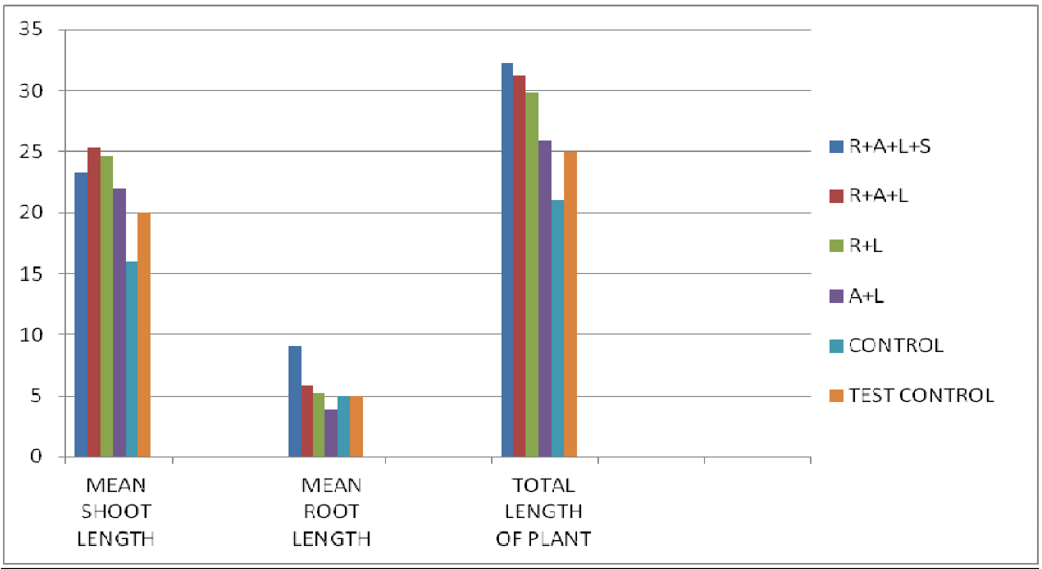
S.no	Types of cultures	Estimation of total chlorophyll mg/gm of sample
1	R+A+L+S	0.123
s2	R+A+L	0.091
3	R+L	0.066
4	A+L	0.040
5	Control	0.050
6	Test control	0.059

Table 5. Protein estimation of the product

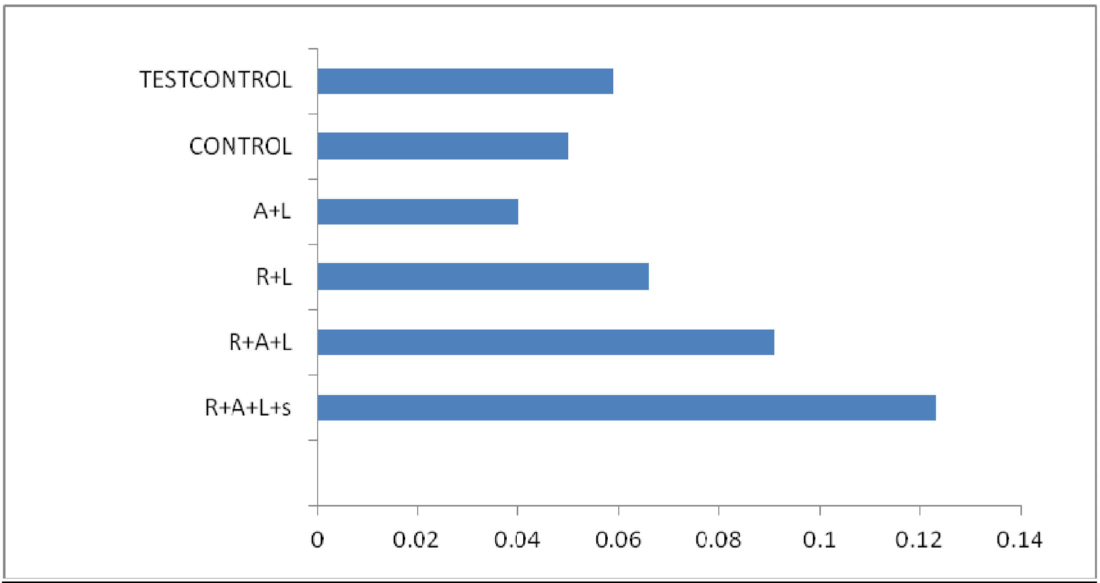
S.NO	Types of cultures	Volume of sample(ml)	Volume of NAOH(ml)	Conc. Of sample(mg/ml)	O.D at 650nm	Volume of BSA(ml)	O.D of BSA at 650nm
1	**R+A+L+S**	1ml	5ml	12mg	0.14	Blank	0.0
2	**R+A+L**	1ml	5ml	10mg	0.13	0.2	0.25
3	**R+L**	1ml	5ml	11mg	0.11	0.4	0.5
4	**A+L**	1ml	5ml	5mg	0.10	0.6	0.77
5	**Control**	1ml	5ml	5mg	0.06	0.8	1.02
6	**Test control**	1ml	5ml	8mg	0.10	1.0	1.34



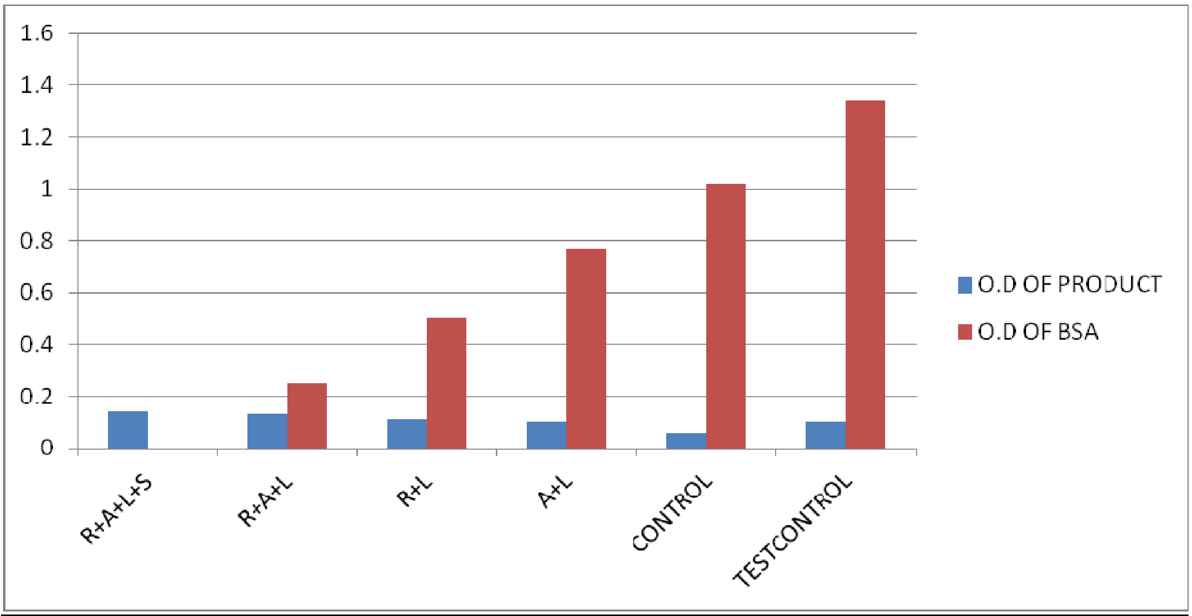
Graph 1. Percent of germination



Graph 2. The average lengths of root, shoot and total length of plantlets



Graph 3. chlorphyll estimation from leaves



Graph 4. Protein estimation of the product

CONCLUSION

So it is better to use a fertilizer containing microbes than a chemical fertilizer. [19,20] As the chemical fertilizers are causing health disorders and even affected the cultivable soils badly, their usage is further regretted and application of biofertilizers and compost with organic waste like kitchen waste are to be encouraged here after for pollution free and healthy life styles of mankind.

Beware of chemicals and save lives, avoid pollution save our mother earth.

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