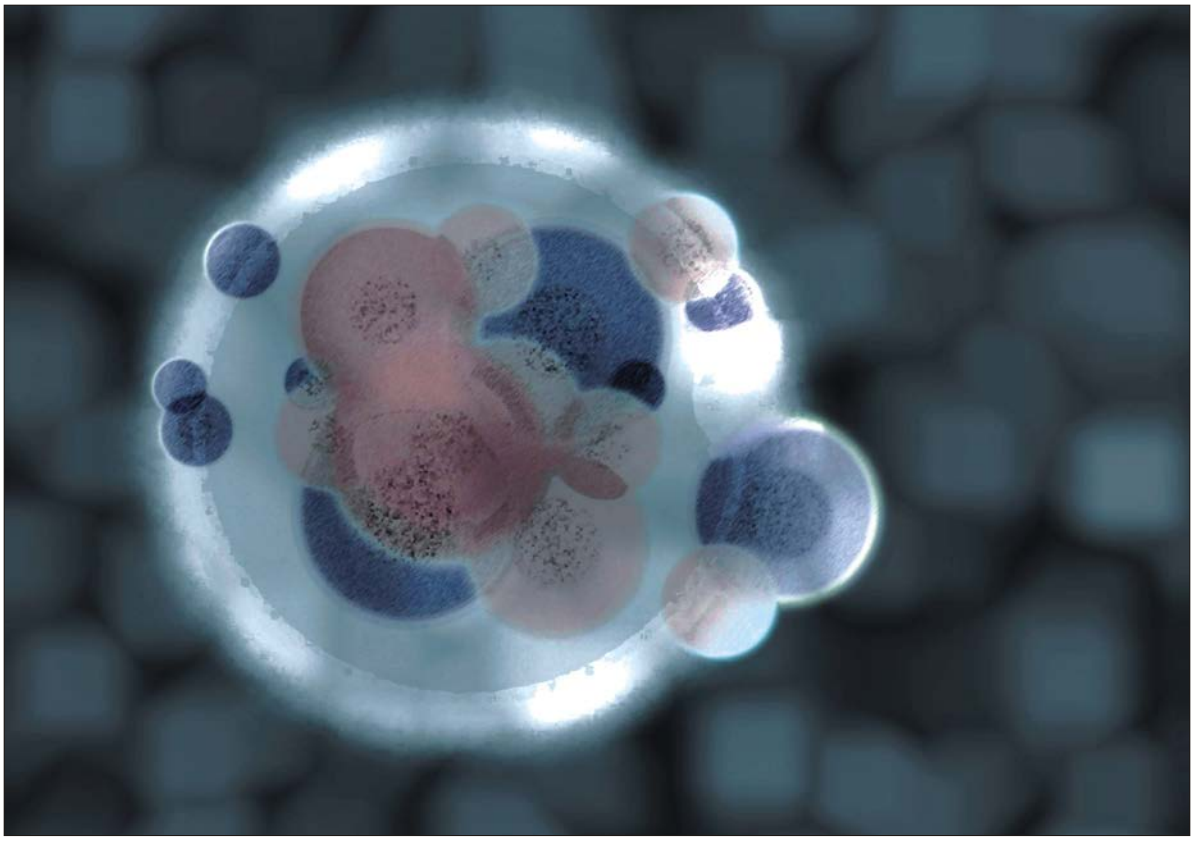

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Novel Instant Organic Fertilizer and Analysis of its Growth Effects on Spinach

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Abstract: The production of novel fertilizers from natural sources has gained importance for the past few years since the continuous use of chemical fertilizers has led to soil pollution, acidification of ground water, destruction of microflora of the soil and in the adverse, has led to emission of methane from uncontrolled dumping activities which inevitably contributes to the green house gases accumulation that can alter global climatic conditions; hence, there is an immediate need to reduce the usage of chemical fertilizers. The present study focuses on the production of Instant Organic Fertilizer (IOF) and its effect over the plant growth (Spinach). A comparative study was performed to analyze the effect of IOF, chemical fertilizer (urea), compost and untreated on the growth of Spinach. The IOF treated spinach showed protein content (73.86 mg mL^{-1}), chlorophyll (32.44 μg mL^{-1}), total phenol (49.84±0.041 mg g^{-1}), 65.56±0.023% (H_2O_2 scavenging activity) and of 65.92±0.22% (DPPH scavenging activity). The present study exposed the efficiency of the economic, eco-friendly and novel IOF in raising high productive and nutritive vegetable crops.

Key words: Instant organic fertilizer, green house gases, chlorophyll, total phenols, antioxidant property, protein content

INTRODUCTION

For sustained agricultural production, use of efficient fertilizer to maintain the soil and plant quality is critical. The application of organic fertilizer has been practiced for more than thousand years in many countries since it provides essential nutrients to plants, improves soil structure, helps in the moisture retaining capacity in various soils and increases microbial activities. Good soil structure makes easier for the plants roots to reach moisture and to absorb the nutrients in the soil (Jedidi *et al*., 2004).

Agricultural and animal wastes are the major raw materials for organic fertilizers that are produced by the process of composting. The composting process is enhanced by the application of certain bacteria and actinomycetes (Moller, 2009). The population of cellulolytic, pectinolytic, lipolytic and amylolytic bacteria are responsible for the hydrolysis of cellulose, pectin, fat and starch and the degradation rate is increased by various hydrolytic enzymes (Shoman *et al*., 2006; Toor *et al*., 2006).

Organically grown food is rich in nutrient value which is not a new concept but organic fertilization are not widely used due to certain limitations like their production time, production cost, labour requirement. Thus, it has reduced their usage and paved way for the use of inorganic/chemical fertilizers. Asia is the world's largest chemical fertilizer consuming continent of about 40% of the total global production. Even in an agricultural nation like India the use of inorganic and chemically synthesized fertilizer is in large proportion. Though organic fertilizers generally contain lower concentrations and release nutrients at a slower rate compared to synthetic fertilizers, organic fertilizers offer a healthier alternative to chemical fertilizers (Rajpaul *et al*., 2004).

The advantage of using these kinds of organic fertilizers is; they provide balanced nutrient supply, facilitates the growth of beneficial microorganisms and helps to suppress certain plant diseases and soil borne diseases (Sary *et al*., 2009; Lowry *et al*., 1951). These kinds of fertilizers should fulfil the needs of green revolution. Thus with these fertilizers high yield/high quality/low cost/low environmental impact crops can be produced.

Well developed countries have promoted high focus in the production of bio fertilizers and organic fertilizers with consideration to the environment. For the under developed countries, these kind of fertilizers from organic wastes may be a boon for their agricultural sector development and may reduce the risk of pollution. But the agricultural use of compost remains is low for several

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reasons like; the product is bulky, making it expensive to transport. The composting process has certain drawbacks like requirement of adequate land area and suitable equipment to operate composting, takes long time to get stable compost product. Therefore, the requirements of raw materials, space and equipment as well as the length of time required for composting usually discourage farmers from making compost by themselves. Hence the main objective of the present work includes the production of time saving, economical instant organic fertilizer and determining its effects on plant growth.

MATERIALS AND METHODS

Instant organic fertilizer (IOF) preparation: The Instant organic fertilizer was prepared by collecting the vegetable and fruit waste collected from the canteen at the university campus. These were sun dried for a week and ground to get a homogenous powder. The newly prepared fertilizer was analysed for its NPK and moisture content.

Experimental set up: Four pots of 5 kg capacity were prepared for each experimental plant; the pot contained a 10 g IOF, 1 g chemical fertilizer (urea), 250 g compost and a control (with out fertilization), respectively. The experimental plant selected was spinach. In each pot 6 seeds were sown and left for germination. The fertilization was done for every 7 days.

Examination of microbial diversity: The increase utilization of chemical fertilizer has diminished the normal microbial flora in soil that plays an essential role in recycling of biomaterials. Hence the soil treated with IOF, compost and chemical fertilizer were analysed for the diversity of bacteria, fungi and actinomycetes population in soil, using dilution plate technique with suitable media like nutrient agar, potato dextrose agar and actinomycetes isolation agar, respectively.

Determination of plant growth promoting activity: Growth promoting activities of soils treated with different fertilizers were tested. The plumule lengths of spinach seedlings were measured initially and were sown in the soils and soon after the development of plumule; it was taken out and soaked in water. After 24 h the plumule length was measured.

Effect of IOF, chemical fertilizer, compost on spinach growth: The plant fertilized with Instant organic fertilizer, compost and chemical fertilizer were analysed for its growth for a period of 45 days. The parameters examined are height, fresh and dry weights, pH, leaf size, diameter, protein concentration, chlorophyll concentration, total phenols and free radical scavenging activity. The values were recorded in triplicates. At harvest time (after 45 days) the leaves were collected and subjected to analysis.

Estimation of protein and chlorophyll: The protein content was estimated by the method of Lowry *et al.* (1951) and Samman *et al.* (2008). The chlorophyll content of the sample was also determined by the method of Arnon (Tan *et al.*, 2009).

Determination of total phenols: The total phenols were estimated by the method of McDonald *et al.* cited by Wu *et al.* (2005). The diluted plant extract was taken in different concentrations and was made up to 1 mL with distilled water. To the samples 2.5 mL of 20% sodium carbonate solution and 0.5 mL of Folin's solution was added and kept for incubation for 40 min. The absorbance was recorded at 725 nm. Tannic acid in different concentrations was used as reference standard. Total phenol values are expressed in terms of tannic acid equivalents.

Determination of hydrogen peroxide (H_2O_2) scavenging activity of plant extract: Hydrogen peroxide scavenging activity of plant extract was determined using a modification of the method of Ruch *et al.* cited by Tejada *et al.* (2009). Solution of H_2O_2 was prepared in phosphate-buffered saline (PBS, pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorbance at 230 nm. Different concentrations of plant extract corresponding to extract stock solution were added in 4 mL distilled water and are then added with 0.6 mL hydrogen peroxide PBS solution. Absorbance of H_2O_2 at 230 nm was determined 10 minutes later against a blank solution containing plant extract in PBS without H_2O_2.

DPPH radical scavenging activity: DPPH radical scavenging activity was carried out by the method of Molyneux (Asal, 2010). To 1.0 mL of 100.0 μM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration was added and incubated in dark for 30 min. The change in colouration was observed in terms of absorbance using a spectrophotometer at 515 nm. One milliliter of methanol

instead of test sample was added to the control tube. Different concentration of ascorbic acid was used as reference.

Statistical analysis: Results calculated from triplicate data were expressed as Means±SD. The data were compared by Least Significant Difference (LSD) test using statistical analysis.

RESULTS

The NPK content in the newly produced Instant organic fertilizer were found to be found to be 1.18, 5 and 3.06 mg g^{-1}, respectively. The moisture content was found to be 3.3%. The pH of the fertilizer was 6.0-6.2. The ash percentage was found to be 11%. These results evident that Instant organic fertilizer produced has required amount of essential nutrients to facilitate plant growth. The spinach seeds soaked in different fertilized soils showed marked difference in plumule length. Table 1 indicates the soil treated with different fertilizer have different growth promoting activity with a good increase in seeds treated with Instant organic fertilizer. Data represented in Table 2 showed that, growth parameters of leaf vegetable spinach were greatly influenced by fertilization treatments. A gradual increase in all the parameters studied (germination period, leaf number, leaf area, plant height, fresh weight, dry weight), progressed with the plant age. Significant differences among the fertilized and control plants were observed. And less difference were found between the plants fertilized with chemical, instant organic fertilizer and compost. Instant organic fertilizer has positive effect on the content of photosynthetic pigments and protein. The sustainable release of nutrients provided the plant to have increased protein and chlorophyll concentration. The content was not much significantly higher in Instant organic fertilizer when compared with chemical treated plant (Table 3). The statistical analysis showed a positive and highly significant, $R^2 = 0.062$ (H_2O_2) and $R^2 = 0.079$ (DPPH) for instant organic fertilizer treated spinach and showed significant differences in phenolic content and high scavenging effect when compared to other fertilized plant (Table 4). These experimental data proved that the newly formulated Instant organic fertilizer is best in all aspects of plant growth (Fig. 1a-d). The inorganic fertilizer also gave the similar results; however, the use of inorganic fertilizer may cause problems in human health and environment.

Table 1: Growth promoting activity of different fertilizer treated soils

Plumule length (cm)	Normal soil	IOF treated soil	Chemical fertilizer treated soil	Compost treated soil
Initial length	0.5	0.9	0.7	0.8
Final length	0.8	1.4	1.7	1.2

Table 2: Effect of different fertilizers on spinach growth for a period of 45 days

Parameters	Normally grown	IOF treated	Chemical fertilizer treated	Compost grown
Germination period	3	3	3	5
Plant height (cm)	17.66±2.41	23.55±3.06	22.55±2.93	22.22±2.69
No. of leaves	7	12	11	12
Leaf diameter (cm)	3.95±0.69	5.43±1.33	5.6±1.38	5.7±1.87
Fresh weight (g)	5.35±1.93	8.46±2.62	8.09±2.62	7.3±2.68
Dry weight (g)	2.84±1.25	3.73±1.52	4.28±1.98	3.21±1.54
Leaf pH	7.8	7.7	8	8.1

Table 3: Effect of different fertilizers on protein and chlorophyll content in spinach

	Normally grown		Instant organic fertilizer treated		Chemical fertilizer		Compost grown	
Day	Protein	Chlorophyll	Protein	Chlorophyll	Protein	Chlorophyll	Protein	Chlorophyll
15th	1.26	9.09	2.07	14.22	1.35	11.65	0.92	9.09
30th	7.06	33.83	6.55	42.51	6.04	40.48	5.49	37.56
45th	11.16	35.02	14.72	44.66	13.36	41.24	12.11	39.73

Protein and chlorophyll concentration is expressed as mg mL^{-1} and μg mL^{-1}, respectively

Table 4: Total phenolics and antioxidant activity of spinach

Parameters	Normally grown	Instant organic fertilizer treated	Chemical fertilizer treated	Compost grown
Total phenolics (mg g^{-1})	43.05±0.020	49 .84±0.041	47.79±0.029	46.70±0.024
H_2O_2 scavenging activity (%)	54.59±0.015	65.56±0.023	60.77±0.020	62.12±0.015
DPPH scavenging activity (%)	51.63±1.57	65.92±0.22	55.55±2.26	60.76±0.41

All data were average (±SD) of three replicates

Fig. 1(a-d): Fertilizer effects on spinach growth. Cultivation was carried out for 45 days; (a) Normally grown spinach, (b) Instant organic fertilizer treated spinach, (c) Compost grown spinach and (d) Chemical fertilizer treated spinach

DISCUSSION

The present study, recorded higher microbial population (bacteria, fungi, actinomycetes) in instant organic fertilizer fertilized soil than with other fertilized soils was clearly depicted in Fig. 3. This may be due to existing temperature and pH in instant organic fertilizer might invite large number of microbes which obviously facilitates the easier degradation of wastes therefore enriching soil properties. Any microbial species release phytohormones and may stimulate plant growth. The microbial diversity in agricultural soil is quite discriminating now days by the continuous application of chemical fertilizers. These data confirms that newly formulated Instant organic fertilizer showed higher growth effects like chemical and organic compost. Chicken manure and farm yard manure, had a synergestic effect on both fresh weight and dry weights of tomato shoots and agricultural compost gave the lowest growth of tomato growth, yield, chemical constituents and mineral nutritional contents and might be due to high pH, losses of leaching and volatilization under soil poor condition. A promotion effect may be due to instant, compost organic and inorganic might be due to the fact that N is a constituent of chlorophyll molecule. The role of natural

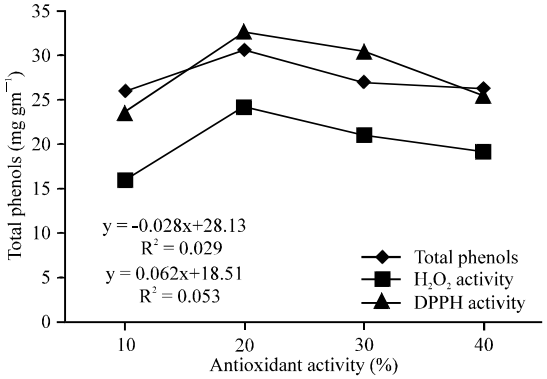

Fig. 2: Relationship between phenol content and antioxidant activity of spinach

dietary antioxidants in disease prevention has been the focus of much investigation. The significant differences are due to the over-fertilization of compost and chemical fertilizer might reduce the health benefits from Spinach. This is an agreement with Mitchell (2007), experimented with tomatoes. Figure 2 shows the relationship between the phenolic content and antioxidant activity in spinach, the accelerated antioxidant activity was associated with high phenolics value. Toor *et al.* (2006) investigated

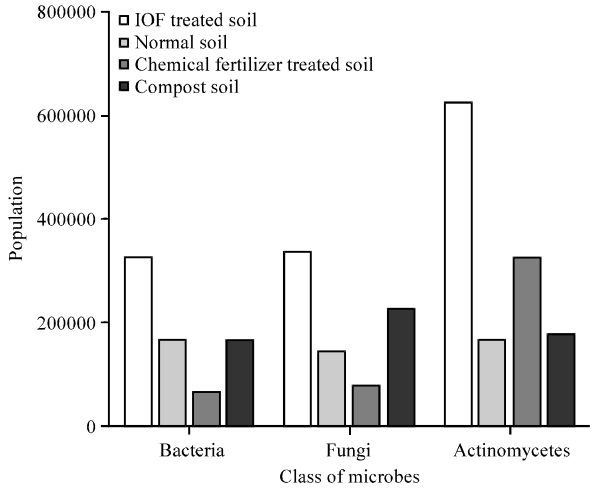

Fig. 3: Enumeration of microbial diversity in soils

three inorganic and 2 organic fertilizer methods to determine if nitrogen availability affects the production of polyphenols in plants. Dauda *et al*. (2005) showed higher level of some individual phytochemicals in organically grown lettuce but not significantly different. No supports was found for higher levels of antioxidants and polyphenols in the conventionally grown vegetables.

CONCLUSION

The instant organic fertilizer used in the study gave the highest results in the physical properties of spinach and also in the protein, chlorophyll concentration and antioxidant activity, equivalent to compost grown spinach. It was therefore to be expected to use Instant organic fertilizer by the farmers since composting operation should have appropriate background and techniques for producing high-quality composts without creating odour and other environmental problems. The main disadvantage of composting is variable costs; which include labour, fuel, electricity and maintenance charges. Hence the study clearly showcases an innovative idea as well as a new approach for the utilization of agricultural waste in the formulation of economical instant organic fertilizer.

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