

Influence of Fresh and Decomposed *Parthenium* and Poultry Droppings on Sesame Yield

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ABSTRACT

Sesame seed is a source of edible oil and is also used as a spice. The seed contains a high percentage of oil which has very good stability due to the presence of natural anti-oxidants. The utilization of waste through recycling is essential for environmental safety, economic stability and ecological sustainability. Several methods have been developed to convert biowastes into organic manure as an alternate source of farmyard manure and a substitute for chemical fertilizers. A pot culture experiment was conducted to assess the effect of fresh, composted, and vermicomposted *Parthenium* and poultry droppings on the yield and quality of sesame. There were 15 treatments, each with three replications. Fresh, composted, and vermicomposted *Parthenium* and poultry droppings were mixed with red soil and sand at 2:1. The maximum number of capsules/plant, weight of grains/plant, and weight of capsules/plant for sesame were with poultry droppings at 35.0 g/pot. The weight of capsules/plant increased when composted *Parthenium* was applied at 35.0 g/pot and 100-grain weight increased when vermicomposted *Parthenium* was applied at 35.0 g/pot. The ascorbic acid content of sesame grains was higher in the N:P:K treatment while the protein content was highest when poultry droppings were applied at 35.0 g/pot.

Keywords: ascorbic acid, organic manure, production, protein, sesame grains

INTRODUCTION

The maintenance of environmental safety and agricultural sustainability is necessary for agricultural production without reducing productivity. Excessive use of chemicals is not only detrimental to crop plants over a long period of time but also makes soil ecologically fragile, losing its productivity together and its economic impact (Deka *et al.* 2003).

As a consequence of the reduced availability of organic materials for agricultural uses, recycling and the use of different organic wastes have received increasing attention (Lobo 1988).

Parthenium hysterophorus, popularly known as congress grass, is famous for its toxic and allergic properties. It causes health problems to both man and domestic animals. It is now widely dispersed and has invaded cultivated fields. *Parthenium* removes a major portion of nutrients and depletes soil fertility while reducing crop yield considerably (Sharma and Gautam 2004). The high concentration of elements (N, P, K, Fe, Mn, Cu and Zn) in composted *Parthenium* has been used to increase the yield of many agricultural crops (Kishor *et al.* 2010). Sivakumar *et al.* (2009) observed an increase in EC, N, P, K and micronutrients (Cu, Zn, Mg and Fe) in all earthworm-worked *Parthenium* composts indicating that the activity of the earthworm *Eisenia fetida*, along with microorganisms, promoted mineralization and made nutrients readily available for plant growth.

Vermicompost (VC) produced by utilizing the straw of boerhavia (*Boerhavia diffusa*), pyrethrum (*Chrysanthemum cinerariaefolium*), henbane (*Hyoscyamus muticus*), fennel (*Foeniculum vulgare* Mill.), chamomile (*Matricaria recutita*), opium poppy (*Papaver somniferum*), coriander (*Coriandrum sativum*), linseed (*Linum usitatissimum*), mustard (*Brassica campestris*), isabgol (*Plantago ovata*), marc of qinghao (*Artemisia annua*) and distillation waste of geranium (*Pelargonium graveolens*), menthol mint (*Mentha*

arvensis), bergamot mint (*Mentha cardiaca*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), garden mint (*Mentha viridis*), lemon grass (*Cymbopogon flexuosus*), citronella (*Cymbopogon winterianus*) and African marigold (*Tagetes minuta*) obtained from the vermicomposting unit of Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow emphasizes the use of VC produced from agro-waste of medicinal and aromatic plants which, apart from providing benefits of supplementing a higher amount of macro- and micronutrients, support better growth/health of plants while also reducing losses from phyto-nematode infestation in crop plants. This strategy will be particularly useful to farmers growing medicinal and aromatic plants who can economically utilize their crop waste (Pandey and Kalra 2010). Earthworm species such as *Eudrilus eugeniae* (Kinberg) are voracious feeders of organic wastes, and their presence has been found to reduce the time required for composting (Prabha *et al.* 2007). Vermicomposting of neem (*Azadirachta indica* A. Juss) was accomplished in 'high-rate' reactors operated at earthworm (*E. eugeniae*) densities of 62.5 and 75 worms/l of reactor volume. The earthworms fed voraciously on the neem compost, converting up to 7% of the feed into VC/day. The growth, flowering, and fruiting of brinjal (*Solanum melongena*) plants with VC had a significantly beneficial impact (Gajalakshmi and Abbasi 2004). Litter of mango (*Mangifera indica*) tree leaves was composted and then converted into vermicast by the action of *E. eugeniae* (Gajalakshmi *et al.* 2004).

Animal manures can be a plentiful source of organic soil amendment although proper management is imperative to prevent adverse environmental effects that can result from the application of manure to soil. Adesodun *et al.* (2005) found that the application of poultry manure to soil increased soil organic matter, and N and P and aggregate stability. The improvement in soil physical properties is

attributed to improvement in soil organic matter content. Improved soil moisture associated with poultry manure is attributed to the mulching effect of organic matter and improved moisture retention and water acceptance as a result of improved soil structure and macro porosity (Aluko and Oyedele 2005).

Vermicomposting and composting are efficient methods for converting solid wastes into useful products. The incorporation of composts and VCs into potting and container media is a potential use for these materials (Hashemimajid *et al.* 2004). Composting may provide a beneficial alternative method for handling poultry litter due to immobilization of nutrients and a reduction in litter volume (Preusch *et al.* 2004).

Sesame is grown in many parts of the world on over 5 million acres (20,000 km²). Sesame is an antique oilseed crop of India, cultivated in 2.5 M ha of land (14% of total area under oilseed crops). However, the productivity of sesame in India is only 335 kg/ha versus its genetic potential of around 2000 kg/ha (Saravanan and Nadarajan 2005).

Sesame seeds contain manganese, copper and calcium, Vitamin B1 (thiamine) and Vitamin E (tocopherol). They are rich in powerful antioxidants (lignans). There are two special lignans present in sesame seeds: sesamin and sesamol. Also present are phytosterols. It is one the most nutritious of seeds and is used culinarily as a spice. Sesame is used in the treatment of anemia, blurred vision and relaxation of the bowel (Saravanan and Nadarajan 2005).

Although sesame is an important oilseed crop for small farmers in many tropical areas of the world, national sesame improvement programs are generally not strong, nor do any international agricultural research centres support sesame improvement. Sesame yields could be greatly improved for the development of agriculture, medicine and industry (Saravanan and Nadarajan 2005).

In the present investigation an attempt has been made to study the effect of fresh, composted and vermicomposted parthenium and poultry droppings (PD) on the yield components and protein and ascorbic acid content of sesame.

MATERIALS AND METHODS

Pot culture experiment to study the efficacy of fresh and decomposed manure

A pot culture experiment was carried out to study the effect of fresh and decomposed parthenium and PD on the yield components and quality of sesame. There were 15 treatments (Table 1) laid out in a completely randomized design each with three replications. The dosages of the manures were as per the recommendations of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for red soil and also based on the nutrient contents of the manures. The NPK levels applied were the recommended dosage for the sesame plant variety used in this experiment.

The fresh, composted and vermicomposted parthenium and PD were assessed for their manurial value in terms of micro- and macronutrients, physico-chemical parameters and microbial population. The decomposed form was significantly different from the fresh form and the improved nutrient value was assessed in this experiment. The manures were then tested for their manurial value in terms of crop growth.

The physical properties like pH and electrical conductivity (Jackson 1973) and bulk density, particle density and pore space (cylinder method) were assessed. The macronutrients assessed were carbon (Walkley and Black 1936), nitrogen (micro-Kjeldahl method; Humphries 1956), phosphorus (colorimetric method; Jackson 1973) and potassium (Piper 1966). The micronutrients, iron, manganese, zinc and copper were also assessed (Jackson 1973). Microfloral population in the compost was determined by the Serial Dilution Plate Technique (Warcup 1950). More details on these methods are explained in the Appendix.

Table 1 List of fresh, composted and vermicomposted parthenium, poultry manure and treatments and their application rates.

Treatment	Treatment detail	Rate (g/pot)
T ₀	Control, 7 kg red loamy soil	
T ₁	Fresh parthenium	35
T ₂	Composted parthenium	26.25
T ₃	Composted parthenium	35.0
T ₄	Composted parthenium	43.75
T ₅	Vermicomposted parthenium	26.25
T ₆	Vermicomposted parthenium	35.0
T ₇	Vermicomposted parthenium	43.75
T ₈	Fresh poultry manure	35.0
T ₉	Composted poultry manure	26.25
T ₁₀	Composted poultry manure	35.0
T ₁₁	Composted poultry manure	43.75
T ₁₂	Vermicomposted poultry manure	26.25
T ₁₃	Vermicomposted poultry manure	35.0
T ₁₄	Vermicomposted poultry manure	43.75
T ₁₅	NPK mineral fertiliser	35:2:23 kg/ha

Each treatment shows the mean values of three replications

SEd Standard Error Deviation

CD Critical Difference

Yield attributes and biochemical parameters studied

The fruits that developed on each plant were counted and recorded as a whole number. The weight of a single fruit was expressed in g. Fruits and grains collected from each plant were weighed and recorded in g. The protein content was estimated following the method of Lowry *et al.* (1951) and ascorbic acid by the method of Sadasivam and Theymoli (1987).

Statistical analysis

The data (standard error of deviation and critical differences) collected from the different studies were subjected to statistical analysis using one way analysis of variance (ANOVA) (Panse and Sukhatme 1978) and group means were compared using *P* at 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Yield and quality attributes of sesame at harvest

The yield components of sesame and its quality, which were observed at 90 DAS, differed significantly among the treatments. The effect of manure on yield parameters (Table 2) and quality (Table 3) were significant.

1. Yield attributes

A significantly higher number of capsules per plant was observed in T₄ (28.00) for *Parthenium* and T₈ (37.33) for PD and the lowest in T₁₄ (3.33). T₃ and T₁₀ increased the capsule weight (0.72 and 0.70 g), whereas the control was only 0.31 g. Maximum values for weight of capsules per plant were noticed in T₄ among *Parthenium* (13.38 g) and T₈ among PD (16.48 g). T₁₄ exhibited a value (2.43 g) lower than the control. Significantly higher grain weight per plant was observed in T₆ (2.48 g) among *Parthenium* and in T₈ (3.34 g) among PD applications compared to lower weight in T₁₂ (0.31 g). The 100-grain weight was maximum in T₆ (0.43 g) and T₁₀ (0.41 g) among *Parthenium*- and PD-treated plants, respectively. A minimum value of 0.20 g was found in the control.

Grain and haulm yield of soybean (*Glycine max*) was highest when 100% VC was used (Reddy and Reddy 1999). Krishnamoorthy and Vajranabaiah (1986) attributed the positive effect of VC to soybean growth to the presence of several plant growth hormones like cytokinins and auxins which have a positive beneficial effect on all yield parameters. As in the present study, Sivakumar *et al.* (1980) observed that the application of compost favourably influ-

Table 2 Impact of fresh, composted and vermicomposted *Parthenium* and poultry droppings on the yield attributes of *Sesamum indicum*.

Treatment	Number of capsules per plant	Weight of a capsule (g)	Weight of capsules per plant (g)	Weight of grains per plant (g)	100-grain weight (g)
T ₀	10.33	0.31	4.38	1.26	0.20
T ₁	21.00	0.65	10.62	1.73	0.28
T ₂	25.67	0.69	12.43	2.23	0.36
T ₃	22.33	0.72	7.75	1.84	0.30
T ₄	28.00	0.58	13.38	2.35	0.42
T ₅	26.00	0.38	7.36	1.30	0.37
T ₆	16.33	0.68	10.44	2.48	0.43
T ₇	16.00	0.57	7.10	1.37	0.31
T ₈	37.33	0.68	16.48	3.34	0.24
T ₉	25.00	0.51	13.15	2.87	0.27
T ₁₀	28.67	0.70	14.45	2.99	0.41
T ₁₁	25.00	0.68	12.01	2.59	0.27
T ₁₂	9.00	0.45	4.50	0.31	0.25
T ₁₃	7.33	0.47	3.48	0.53	0.31
T ₁₄	3.33	0.43	2.43	0.56	0.23
T ₁₅	11.67	0.46	3.74	0.63	0.26
SEd	1.74	0.04	0.66	0.15	0.02
CD (5%)	3.56**	0.07**	1.34**	0.31**	0.04**

Each treatment shows the mean values of three replications; SD = standard deviation; CD = critical difference

enced the fruiting points, boll number and the boll setting per cent of cotton which may be due to the slow decomposition and immobilization of nutrients from compost. Bachthaler and Wonneberger (1974) obtained higher vegetable yields for celery (*Apium graveolens*) and potato (*Solanum tuberosum*) when PD was applied. Adediran *et al.* (2003b) compared PD, household, market and farm waste and found that PD at 20 t/ha had highest nutrient contents and mostly increased the yield of tomato (*Lycopersicon esculentum*) and the macro- and micronutrient content of soil. Akande and Adediran (2004) found that PD at 5 t/ha significantly increased tomato and dry matter yield, soil pH, N, P, K, Ca and Mg content and uptake. The yield of potato and the average weight of potato tubers were significantly higher in plots treated with VC (based on paddy straw and cattle dung). This may be attributed to increased bioavailability of P by the application of an organic amendment in the form of VC (Ansari 2008). Treatment with VC produced from agro-waste of medicinal and aromatic plants significantly increased the fresh/dry root and shoot weight, fruit number and fruit weight of tomato plants (Pandey and Kalra 2010). Five tonnes of VC together with 50: 50: 50 N, P₂O₅, K₂O kg/ha gave the highest vegetative yield as well as nutrient uptake, followed by 2.5 t/ha of VC + NPK, implying a synergistic effect in the combined application of VC and chemical fertilizers in amaranth (*Amaranthus tricolor* L.) production (Preetha *et al.* 2005).

2. Protein and ascorbic acid content

Protein content was highest in T₂ (16.07 mg/g) among *Parthenium* and T₈ (15.71 mg/g) among PD treatments compared to the control (9.57 mg/g). The ascorbic acid content was higher in T₂ (548.93 mg/g) among *Parthenium* treatments and T₁₅ (572.80 mg/g) had a higher value than in PD-treated crops. T₇ had the lowest value (334.13 mg/g).

Ascorbic acid content in litchi (*Litchi chinensis*) fruits increased after the application of NPK (Hasan and Chattopadhyay 1993). The crude protein content of tomato fruits increased when the recommended dose of N was used in the form of PD (Prabakaran and James 2003). Maximum plant growth, carbohydrate, protein and chlorophyll were achieved for *Andrographis paniculata* when grown in garden soil amended with coir pith VC (Vijaya *et al.* 2008). The fat content of okra (*Abelmoschus esculentus*) fruits was maximum with vermiwash and VC (derived from cow dung and paddy straw) followed by VC and vermiwash while the pro-

Table 3 Impact of fresh, composted and vermicomposted *Parthenium* and poultry droppings on the protein and ascorbic acid content of *Sesamum indicum* at 90 DAS.

Treatment	Protein	Ascorbic acid
T ₀	9.57	405.73
T ₁	9.83	381.87
T ₂	16.07	548.93
T ₃	11.68	425.07
T ₄	13.73	501.20
T ₅	11.28	429.60
T ₆	10.98	429.60
T ₇	13.24	334.13
T ₈	15.71	358.00
T ₉	10.38	405.73
T ₁₀	12.30	477.33
T ₁₁	12.85	501.20
T ₁₂	10.45	525.07
T ₁₃	12.97	429.60
T ₁₄	10.78	477.33
T ₁₅	12.78	572.80
SEd	0.65	59.09
CD (5%)	1.34**	120.69**

Each treatment shows the mean values of three replications; SD = standard deviation; CD = critical difference

tein content of fruits was maximum in vermiwash and VC followed by VC and cow dung (Ansari and Kumar 2010). The biochemical qualities of the fruits grown in vermiwash and VC had higher nutrient quality, which may be attributed to the presence of plant growth promoters like gibberellins, cytokinins and auxins (Krishnamoorthy and Vajranabai 1986). Vermiwash is a major contributor of micro-nutrients to soil. VC and vermiwash are also rich in certain metabolites and vitamin B or provitamin D, which also enhance plant growth (Lalitha *et al.* 2000; Ansari 2008a, 2008b). According to Lalitha *et al.* (2000), the application of organic fertilizers positively affects okra growth and production. Soil enriched with VC provides additional substances that are not found in chemical fertilizers and provide macro-nutrients and micronutrients in amounts required by plants (Kale 1998; Ansari and Ismail 2008). The present results are in agreement with those obtained by earlier workers (Lalitha *et al.* 2000; Ismail 2005; Ansari 2008a, 2008b; Ansari and Ismail 2008). Therefore organic farming provides many advantages such as the elimination of the use of chemicals in the form of fertilizers or pesticides, the ability to recycle and regenerate waste into productive forms of income-generating substrate and improve soil, plant, animal and human health while creating an ecofriendly, sustainable and economical bio-system model (Ansari and Ismail 2001).

CONCLUSIONS

This study shows that the quality and production of sesame seeds could be improved by the application of decomposed organic manure. The decomposition of organic material improves its quality in terms of micro- and macronutrients and compounds essential for plant growth. Improved growth results in better yield and quality of sesame products.

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APPENDIX

SOIL PHYSICAL PROPERTIES (Jackson 1973)

1. pH and Electrical Conductivity

The pH and Electrical Conductivity of samples were measured using ELTOP digital pH meter (3020) and Delux conductivity meter – 601 E respectively.

Moisture content

Principle

Soil moisture content is determined by drying a known quantity of soil sample in an electric oven at 105°C to 110°C and finding out the loss in weight.

Materials required

(i) Moisture bottle / aluminium tin; (ii) Balance; (iii) Desicator.

Procedure

A clean and empty moisture bottle or aluminium tin with lid was placed separately in an electric oven and kept it at 105°C for 15 min. The stopper or lid was replaced, the moisture bottle or aluminium tin was removed, cooled in a desicator, weighed accurately and the weight was recorded. The moisture bottle or aluminium tin was filled to about two third of its capacity with soil sample. Closed with stopper / lid and weighed quickly. Removed the stopper / lid and kept it in the oven at 105°C for about 8 h. After the expiry of time, the moisture bottle / aluminium tin was removed, cooled it in a desicator and weighed quickly. Calculated the loss in weight and expressed the moisture content on oven dry basis.

Calculations (weight all in g)

Weight of empty moisture bottle = a

Weight of the moisture bottle + moist soil sample = b

Weight of moist soil alone = (b-a)

Weight of the moisture bottle + soil sample after drying in the oven = c

Weight of moisture in the soil = (b-c)

Percentage of moisture in the soil sample on oven dry basis = $\frac{(b-c)}{(c-a)} \times 100$

2. Determination of Bulk Density, Particle Density and Pore Space (Cylinder method)

Bulk density is the mass per unit volume of soil including pore space. The normal range of bulk density in most soils is from 1.02 to 1.8 g/ml. The bulk density of coarse textured soils will be on the higher side while organic soils will have lower bulk density. Particle density is the density of soil solids which is normally around 2.75 g/ml. Porosity is that fraction of soil volume not occupied by solid particles.

Principle

The bulk density and per cent pore space were determined from the apparent and true volumes of the soil measured by adding a known quantity of water to a measuring cylinder containing a weighed quantity of soil.

Materials required

(i) Measuring cylinder (100 ml) with a glass stopper; (ii) Balance.

Procedure

- Exactly 20 g of soil sample was weighed and transferred in small quantities at a time to a 100-ml measuring cylinder with glass stopper by gently tapping the cylinder. After completely transferring the soil the volume was noted.
- A known volume of water (50 ml) was added along the sides of the cylinder using pipette till the entire soil mass was completely soaked. (There should be at least 5 ml of water above the soil surface after the addition of water. Care should be taken to see that the final volume of soil and water is below 100 ml mark).
- The cylinder with soil and water was kept in an undisturbed condition for at least 30 minutes so that the entire pore space is completely filled with water.
- The final volume of soil plus water was noted after the expiry of time and the bulk density, particle density and pore space were calculated.

Calculations (weight in g, volume in ml)

Weight of the soil taken = W (20)

Volume of the soil taken = V_1 ml

Volume of water added = V_2 ml

Volume of soil + water = $V_1 + V_2$ ml

Volume of soil + water at the end of experiment = V_3 ml

1. Pore space volume = $(V_1 + V_2) - V_3$ ml

2. Per cent pore space = $\frac{(V_1 + V_2) - V_3}{V_1} \times 100$

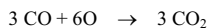
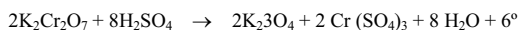
3. Bulk density = $\frac{\text{Weight of soil}}{\text{Volume of soil}} \text{ Mg m}^{-3}$

4. Particle density = $\frac{\text{Weight of soil}}{V_1 - \text{Pore space volume}} \text{ Mg m}^{-3}$

ESTIMATION OF CARBON (Walkley and Black 1936)

Principle

Organic carbon present in organic matter is oxidised by chromic acid ($K_2Cr_2O_7$) in the presence of concentrated H_2SO_4 . Potassium dichromate on reaction with H_2SO_4 provides nascent O_2 which combines with carbon and form CO_2 . The H_2SO_4 enables easy digestion of organic matter by rendering heat of dilution only a certain quantity of chromic acid is used for oxidation. The excess of chromic acid left unused by organic matter is determined by titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator.



Reagents

1. 1N potassium dichromate 12.2 ($K_2Cr_2O_7$): Exactly 49.04 g of $K_2Cr_2O_7$ was dissolved in 1 L of distilled water.
2. Diphenylamine indicator: 0.5 g of diphenylamine was dissolved in 20 ml of water and 100 ml of con. H_2SO_4 was added.
3. 0.5 N Ferrous sulphate or Ferrous ammonium sulphate: 39.0 g of Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) was dissolved in 800 ml of distilled water. 20 ml of con. H_2SO_4 was added and made up the volume to one litre.
4. Con. H_2SO_4 .
5. Phosphoric acid: Orthophosphoric acid (85%).

Procedure

1. 0.5 g of soil was weighed (passed through 0.2 mm sieve) and transferred it to a 250 ml / 500 ml conical flask.
2. 10 ml of 1N $K_2Cr_2O_7$ was added and mixed well by swirling the flask.
3. Then 20 ml of con. H_2SO_4 was added and mixed by gentle rotation for one minute to ensure complete contact of the reagent with the soil.
4. Allowed the contents to stand for 20 – 30 min.
5. The flask was kept preferably on an asbestos sheet to avoid burning of table due to intense heat.
6. The reaction mixture was diluted with 200 ml distilled water.
7. 10 ml of phosphoric acid and 1 ml of diphenylamine indicator were added.

Titrate the solution with 0.5 N ferrous ammonium sulphate. The colour was dull green at the beginning and then shifted to a turbid blue as the titration proceeded. The end point was very sharp. At the end point the colour sharply shifted to a brilliant bright green.

The organic carbon content in the sample was determined using the formula:

$$\text{Per cent organic carbon} = \frac{(B.V. - S.V.) \times 10 \times 0.003 \times 100}{B.V. \times W}$$

where

B.V. = Titre value of blank (ml)

S.V. = Titre value of sample (ml)

10 = Quantity of 1N Potassium dichromate used (ml)

0.003 = One ml of 1N $K_2Cr_2O_7$ used (conversion factor)

W = Weight of the sample (on dry weight basis)

$$\text{Organic matter} = \text{Organic carbon} \times 1.724$$

ESTIMATION OF NITROGEN (Micro KJELDAHL method) (Humphries 1956)

Principle

A known weight of the powdered sample is treated with diacid mixture so as to oxidize the organic matter and bring the mineral elements into solution.

Materials required

- i. 100 ml conical flask; (ii) 250-ml volumetric flask; (iii) Funnel; (iv) Sand bath; (v) Diacid mixture; (vi) Boric acid 2%; (vii) N/10 sulphuric acid; (viii) Bromocresol green – methyl red double indicator; (ix) Sodium hydroxide 40%.

Procedure

- One g of sample was transferred into a 100-ml conical flask.
- 15 ml of diacid mixture (sulphuric acid and perchloric acid in the ratio of 5: 2) was added and covered the mouth of the flask with a funnel. Digested the contents of the flask over a sand bath till a clear solution was obtained.
- The contents of the flask were transferred into a 250 ml volumetric flask using minimum quantity of water and made up the volume to 250 ml.
- 10 ml of the diacid extract was pipetted out into a distillation flask. 25 ml of 2% boric acid was pipetted out into an ice tumbler / beaker and two drops of double indicator (bromocresol green methyl red) was added and kept it at the delivery end of the distillation set.
- 10 ml of 40% sodium hydroxide was added to the distillation flask and distilled the contents and the distillate was collected in 2% boric acid.
- Tested the completion of distillation with a moistened red litmus paper.
- Absence of blue colour indicated that all ammonia had been distilled. The delivery tube was washed with distilled water and the washings were collected in ice tumbler. Titrated the contents with N / 10 sulphuric acid.

The end point was the change of colour from green to pinkish.

Calculations (weight in g, volume in ml)

Weight of the sample taken = 1

Volume of diacid extract prepared = 100 ml

Volume of diacid extract pipetted out for analysis = 10

Volume of N / 10 H_2SO_4 consumed = X

1 ml of N / 10 H_2SO_4 = 0.0014 g of N

This is present in 10 ml of the diacid extract

$$\text{Therefore in 250 ml} = \frac{X \times 0.0014 \times 100}{10} \times \text{g of N}$$

$$\text{This is present in 1.0 g of the sample in 100 g} = \frac{X \times 0.0014 \times 100 \times 100}{10} \times \text{g of N}$$

$$\text{Percentage of nitrogen on moisture = free basis} = \frac{X \times 0.0014 \times 100 \times 100}{10} \times \frac{100}{(100 - M)}$$

where M = moisture content of the sample.

ESTIMATION OF PHOSPHORUS (COLORIMETRIC METHOD) (Jackson 1973)

Principle

When vanadomolybdate and phosphate radical react in nitric acid medium a heteropoly compound is formed which is yellow in colour. The intensity of yellow colour is proportional to the amount of phosphorus in the sample. By reading the intensity of yellow colour produced in a spectrophotometer at 400 – 490 nm the amount of phosphorus in the sample is determined.

Materials required

(i) volumetric flask 25 ml; (ii) pipette; (iii) spectrophotometer; (iv) Barton's reagent prepared by dissolving 25 g ammonium molybdate in 400 ml water and mixing this with a solution containing 1.25 g ammonium meta vanadate dissolved in 300 ml concentrated nitric acid and making up the volume to 1 L.

Procedure

5 ml of the triple acid extract was pipetted out into a 25 ml volumetric flask.

5 ml of Barton's reagent was added, shaken well and made up the volume to 25 ml and made it a homogenous solution. Allowed to stand for about 30 min for the colour to develop. The intensity of yellow colour developed was read in a spectrophotometer at 470 nm. By referring to the standard curve prepared calculated the phosphorus content of the sample.

Calculations (weight in g, volume in ml, concentration in ppm)

Weight of sample taken = w

Volume of triple acid extract prepared = V

Aliquot taken for colour development = 5 ml

Concentration of the solution as read from the spectrophotometer = x

$$\text{Therefore P content on moisture free basis} = \frac{x}{10^6} \times \frac{25}{5} \times V \times \frac{100}{1w} \times \frac{100}{(100 - M)}$$

where M = moisture content of the sample.

ESTIMATION OF POTASSIUM (Piper 1966)

Principle

When liquid samples containing potassium is burnt in the flame, K emits photons characteristics of its wave length. The intensity of the emission is proportional to the K content. By measuring the intensity in a flame photometer the K content is determined.

Materials required

(i) Ammonium hydroxide; (ii) Flame photometer; (iii) Volumetric flask 25 ml; (vi) pipette.

Procedure

- 5 ml of triple acid extract was pipetted out into a 25 ml volumetric flask and 5 ml of ammonium hydroxide was added.
- The volume was made up to 25 ml with water.
- The content was transferred to an injection vial and fed to a flame photometer after adjusting the flame photometer to read zero with distilled water and 100 with 100 ppm K solution.
- The meter reading was noted and calculated the K content by referring to the standard curve prepared.

Calculation (weight in g, volume in ml, concentration in ppm)

Weight of sample taken = 2

Volume of triple acid extract prepared = 250

Volume of aliquot pipetted = 5 ml

Volume of the solution made up = 25 ml

Concentration of K as read from standard curve = A

$$\text{Content of potassium in the sample on moisture free basis} = \frac{A}{10^6} \times \frac{25}{5} \times 100 \times \frac{100}{1W} \times \frac{100}{(100 - M)}$$

where M = moisture content of the sample.

DETERMINATION OF IRON, MANGANESE, ZINC AND COPPER (Jackson 1973)

Several extractants have been tried to extract and estimate the available micronutrient status of the soil. Of these, diethylene triamine penta acetic acid (DTPA) was found to be the most suitable extractant for estimating the content of available Zn, Cu, Mn and Fe by the use of atomic absorption spectrophotometer.

Principle

DTPA forms stable complexes with Zn, Cu, Fe and Mn. Its capacity to complex each of the micronutrient cation is 10 times its atomic weight.

Materials required

(i) DTPA 0.005 M; (ii) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ – 0.01 M solution; (iii) Triethanolamine : 0.1 M solution. TEA suppresses the solubility of CaCO_3 which will release the occluded micronutrients (unavailable to the plant).

The DTPA extractant solution was prepared by dissolving 13.1 ml of TEA, 1.967 of AR grade DTPA and 1.47 g of CaCl_2 in 100 ml glass distilled water. The contents were allowed for some time so that the DTPA will dissolve and then diluted to about 900 ml. The pH of the solution was adjusted to 7.3 ± 0.05 with 1 : 1 HCl by stirring and the volume made up to 1 L.

Preparation of standard solution

a) Zinc

0.439 g of AR grade $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was dissolved in 200 ml glass distilled water in a beaker to which 5 ml of 1:5 H_2SO_4 is added. The contents are transferred to a one litre volumetric flask and the volume made up. This gives 100 ppm of Zn. From this stock solution working standards of 0, 0.1, 0.2, 0.4 and 0.6 $\mu\text{g} / \text{ml}$ (ppm) solutions were prepared.

b) Iron

0.702 g of AR grade ferrous ammonium sulphate dissolved along with 5 ml of 1:5 H_2SO_4 and made up to 1 litre gives 100 ppm of Fe stock solution. From this stock solution various concentrations of working standards viz., 0, 1, 2, 4, 6 ppm were prepared.

c) Manganese

0.288 g of AR grade potassium permanganate was dissolved in 300 ml of glass distilled water. To this 20 ml of conc. H_2SO_4 was added and warmed to 60°C . Then oxalic acid was added drop by drop to make the solution colourless. The contents were cooled and made up to 1 litre which gives 100 ppm stock solution. From this working standards of 0, 1, 2, 4, 6, 8 ppm were prepared.

d) Copper

0.392 g of AR grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in glass distilled water and made up to 1 litre gives 100 ppm of Cu. From this working standards of 0, 1, 2, 4, 6 ppm solutions were prepared.

From the working standard solutions, the standard curve was prepared for each nutrient. While preparing the standard start from the lowest concentration just after standardising the instrument with blank solution. In between two standard solutions, introduced the blank and ensured that there is no change in the zero point. Prepared a graph by plotting the absorbance values against concentrations. Estimation of the nutrient concentrations.

- Transferred 10 g of air dried soil sample to 150 ml conical flask / polythene bottle and added 20 ml of the DTPA extractant solution.
- Closed the bottle and shaken for 2 hours in a horizontal shaker.
- Filtered through whatman No. 42 filter paper.
- Take a reading of the filtrate in the atomic absorption spectrophotometer and by referring to the standard curve calculate the concentration of each micronutrient in the sample. Shaking time, concentration and pH of the DTPA extractant and temperature will influence the quantity of nutrients extracted.

CARBON : NITROGEN RATIO

C : N ratio was calculated by dividing the percentage of carbon with the percentage of nitrogen.

CARBON : PHOSPHORUS RATIO

C : P ratio was calculated by dividing the percentage of carbon with the percentage of phosphorus.

MICROFLORAL POPULATION

1. Potato Dextrose Agar Medium (Riker and Riker 1936)

Composition

Potato tubers (peeled): 100 g; Dextrose: 5.0 g; Tap water: 1000 ml; pH: 6.5; Agar: 15.0 g

Preparation

Well sliced potato tubers were cooked for 15 min in 250 ml tap water over a water bath. After cooling, the slices were macerated and filtered through two layers of cheese cloth. The volume of the extract was made up to 500 ml with water. For a quantity of 900 ml of water, 100 ml of potato extract was used.

2. Serial Dilution Plate Technique (Warcup 1950)

A quantity of 1.0 g of homogenised sample was weighed and dropped into an Erlenmeyer flask containing 100 ml of sterile water. The flask was placed over a gyratory shaker for ten min. One ml quantity of the solution was withdrawn using a sterile pipette and transferred to 9 ml water blank taken in a test tube. The content of the tube was mixed well. Using 9.0 ml water blanks, serial dilution of the sample was made until a dilution of 10^{-5} was reached. From the last dilution (10^{-5}), one ml quantity of the sample was withdrawn and transferred to sterile Petri dishes. Each plate was added with 20 ml of the medium, rotated well to have uniform spread of the medium. The plates were incubated at 28°C in an incubator for 5-7 days.