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RESEARCH ARTICLE

BIOCHEMICAL ANALYSIS OF PLANT GROWN IN DIFFERENT COMPOSTS

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ABSTRACT

The present study reveled the importance of keratinous compost was significant resulted in to a high valued product acceptable in agricultural, as suitable organic nitrogen source obtained from hair and feather compost. The Application of hair and feather composts improves soil fertility along with other soil properties and could be an attractive natural fertilizer for crops. This method could be an alternative to farm composting to produce a community oriented and eco-friendly compost. The *C. roseus* Plant grown in soil mixed with composts like hair, feather, mulberry leaves of debris compost, cow dung and urea fertilizer used as compared to control one without compost used. The effect of different composts on the biochemical contents of carbohydrates, proteins, amino acid, nucleic acid, indole acetic acid, chlorophyll, carotenoids, phenol, proline, hydrogen peroxide, ascorbate and glutathione were studied in the leaves of *C. roseus*. The treated compost *C. roseus* plant observed significantly increases in hair and feather compost and gradually decreases in debris of mulberry leaves, cow-dung and urea and control plant.

INTRODUCTION

The different composts like hair and feather, debris of mulberry leaves, cow dung, and urea fertilizer applied *to C. roseus* plant. The chosen to comparative study and effect of keratinous compost in related to other composts with reference to growth and biochemical analysis. It is identified with the scientific names of *Catharanthus roseus, Vinca rosea* and *Lochnera rosea* (Omidbeigi 2000, Hormok 1978). Almost all plants of the *Apocynaceae* family contain various kinds of alkaloids or glycosides (Omidbeigi 2009). In the conducted researches on the application of biological fertilizers on medicinal plants, it has been observed that maximum amounts of active compounds have been achieved under these conditions (Kapoor *et al*., 2002, Anwar *et al*., 2005). *C. roseus* plant were grown in pots with different composts like, hair, feather, mulberry, cow dung and urea as a chemical fertilizer and observations were made on two sets of pots, each set consisting of three replicates were grown for the present studies. Burtt and Ichida, (1999b) explored the enzymatic capability of the feather degrading *B. licheniformis* to hasten the composting of dead chickens or feather waste, could be an economical and environmentally safe method of recycling these organic materials into fuel pellets and high-nitrogen fertilizers. Alternatively, *B. licheniformis* could be used to degrade beta keratin into small proteins and amino acids that would provide a low-cost, highly digestible protein additive to livestock and fish food. Feathers soaked in an inoculum of *B. licheniformis* and *Streptomyces* degraded more quickly and more completely (Ichida *et al*., 2001). Inoculation of feather waste could improve composting of the large volume of feather waste generated every year by poultry farms and processing plants. According to Gea *et al*. (2005), hair residue could not be composted alone, but combined with other residues in order to find conditions that favor the composting process. Hair residue was thus mixed with de-linking sludge, an industrial residue that had been successfully composted. Composting is an aerobic process, which requires oxygen to stabilize organic wastes, optimal moisture and enough free air space (FAS) (Haug, 1993). C/N ratio is also important and often used as to design criteria (Haug, 1993). Temperature plays an important role in the composting process; it is routinely chosen as the control variable, because it is an indicator of the biological activity of the material. Composting process is usually carried out within the thermophilic range of temperature permitting the disinfection of the final product (Salter and Cuyler, 2003). Composting of residual hair seems to require the presence of a co-substrate for composting and nitrogen conservation. Recent works have been published on the biodegradation of animal wastes using specific microbial populations (Gousterova *et al*., 2005). Tiquia *et al*. (2005) obtained 50% carbon conversion, during the composting wastes from the poultry industry with high nitrogen content. This indicates the high biodegradability of protein of animal origin under composting conditions. According to Lacey *et al*. (2006) the Poultry litter is perhaps one of the most valuable and appreciated by-products that a poultry farm generates. With the rising cost of commercial fertilizers, poultry litter becomes a more valuable and sought after commodity for crop and forage production. Primitive feathers first appeared on Dinosaurs and microorganisms have evolved to degrade that most recalcitrant protein and β- keratin. Recently little research has focused on this essential application of microbial enzymology. According to Jensen (1995), an every American consumes over 36 kg of chicken/year and the US poultry industry produces up to 815 million kg of waste feathers annually. Disposal of dead poultry and waste feathers is a major concern for processing plants and poultry farms, where mortality is typically about 0.1% and can commonly reach 0.25% per day (Holmes, 1993; Collins, 1995). Nitrogen is also the nutrient that has received the most attention in compost process, as it could be lost significantly during the composting process. Recent studies have showed that about 20-70% of the initial N of the initial feedstock could be lost due to ammonia volatilization, leaching, and

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ru mnoff during composting (Rao Bhamidimarri and Pandey, 1996; Tiquia and Tam, 2000a). These losses not only reduce the value of the composted product as an N fertilizer, but they could also lead to serious environmental pollution (Kirchmann and Lundvall, 1998). Tanzania is however, endowed with a large number of livestock such as, cattle, goats, sheep, pigs, donkeys and poultry. According to Kimbi *et al*. (1999) animal manure output in mainland Tanzania is about 14 million tons per year. If it is assumed that average N content of animal manure is 0.7% of the total N from the manure is 94,500 tons. This is about four times the amount of nitrogenous fertilizers used in the country in 1980. Efficient use of animal manure could therefore alleviate the problem of declining land productivity in most parts of Tanzania. Irrespective of the enormous manure production potential, very little amount of the available animal manure is being utilized for crop production. Kimbi *et al*. (1999) observed that in extensive livestock grazing system only about 1% of farmers apply animal manure on land, indicating serious under utilization of such resources. This is largely due to lack of scientific basis for advising farmers on aspects such as, appropriate application rates, storage techniques and application methods (Gabriel, 1998). Schueter (1989) found that various types of agricultural/municipal wastes suppress different types of soil-borne plant diseases by making plants more vigorous and better able to withstand attack**.** Muhammad *et al*. (2001) also observed that sawdust composted soil, reduced incidence of seedling blight of *Parkia biglobosa* caused by *Fusarium solani* ranged from 30% to 74.2%. While, rice-husks composted soil reduced in the incidence of wilting of *P. biglobosa* caused by *F. solani* ranged from 31.4% to 70.3%.

MATERIALS AND METHODS

Estimation of carbohydrate fractions

One g fresh leaf material was homogenized with ethanol. The homogenate from each treatment was taken into separate centrifuge tube. The tubes were heated on boiling water bath for 5 min. After cooling, the contents were centrifuged at 3,000 rpm for 10 min. The supernatant from each treatment was collected in clean and dry test tube. The residue was re-extracted with 5 ml of 70% ethanol (v/v) and again centrifuged. The centrifugation process was repeated thrice. The ethanol extract from each tube was pooled into their respective tube and the volume was reduced on hot water bath. The concentrated supernatant was used for the estimation of total sugars, reducing sugars and non-reducing sugars. An aliquot of ethanol extract from each treatment was mixed with 5 ml of saturated lead acetate to precipitate proteins. Later, 5 ml of saturated aqueous disodium phosphate was added to precipitate the excess lead acetate. About, 0.2 g of activated carbon was added to it and the contents were shaken to remove colored compounds. The solution was filtered and was made up to 20 ml. This filtrate was used for the estimation of total sugars, reducing sugars and non-reducing sugar.

Estimation of total sugars

A total sugar was assayed as per the method prescribed by Yoshida *et al*. (1976). An aliquot from the alcohol extract was evaporated to dryness in test tube on water bath at 60^0C. The lipids and pigments were removed by washing the evaporated residue repeatedly with diethyl ether. After the ether treatment, residue was dissolved in a small volume of 40% ethanol (v/v). This was used for the estimation of total sugars by anthrone reagent. 5 ml of anthrone reagent (200 mg of anthrone dissolved in 100 ml of concentrated sulphuric acid) was added to 1 ml of each sample. A blank was prepared by using 1 ml of 40% ethanol instead of the extract. The tubes were heated on a boiling water bath for 10 min and were cooled. The absorbance of the resultant brown colored product was measured at 630 nm using spectrophotometer. The total sugar was estimated as D-glucose equivalents. The amount of glucose was calculated using the standard curve prepared by using known amount of D-glucose. The amount of total sugars was expressed in mg/g fresh weight.

Estimation of reducing sugars

Dinitrosalicylic acid (DNS) method was used to assay the reducing sugars. 1ml of DNS reagent (1 g of dinitrosalicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphate were dissolved in 100 ml of 1% NaOH) was added to 1ml of ethanol extract and 3 ml of distilled water. A blank was prepared by adding 1 ml of the reagent to 4 ml of distilled water. Each tube was placed in a boiling water bath for 5 min and cooled to room temperature. The absorbance was read at 540 nm against the blank. The amount of glucose in the sample was calculated with the help of standard graph. The amount of glucose is expressed in mg/g fresh weight.

Estimation of Non-reducing sugars

The amount of non-reducing sugars was calculated as per the formula (Loomis and Shull, 1973) given below: Non reducing sugars = (Total sugars – free reducing sugars) × 0.95. The amount of non-reducing sugars was expressed in milligrams/ gram fresh weight.

Estimation of starch

Starch was estimated as per the method of Mc Creadly *et al*. (1958) from the residue left after alcohol extraction. Starch was solubilized from the residue with 52 % perchloric acid (v/v) for one hour and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was taken and made up to a known volume. 1 ml of perchloric acid extract was diluted to 5 ml with distilled water and 10 ml of freshly prepared anthrone reagent was added in cold. The test tubes were rapidly cooled, shaken and then absorbance was measured at 630 nm in spectrophotometer. Simultaneously, the blank was run without starch. The amount of glucose was calculated from the standard curve prepared by using known amount of glucose. Starch content was expressed in milligrams/gram fresh weight.

Estimation of soluble Proteins

One gram of fresh leaf material was taken and ground with a pestle and mortar in 10 ml of phosphate buffer. The homogenate was centrifuged at 5,000 rpm for 15 minutes. The supernatant was used for the protein estimation. Bradford method (1976) was used for protein estimation. 5 ml of Coomassie Brilliant Blue G-250 (100 mg of Coomassie Brilliant Blue G-250 was dissolved in 50 ml of 95% ethanol). 100ml of 85% phosphoric acid was added to this. This mixture was diluted to 1 liter with distilled water. The dye was filtered to remove undisolved particles and stored in cold conditions was added to each tube containing 1 ml of experimental samples. A tube with 1 ml water instead of protein sample served as blank. Tubes were mixed well and allowed for colour development for 5 minutes. Absorbance was read at 595 nm. The protein in the experimental sample was calculated using the BSA protein standard.

Estimation of Amino acids

One gram of fresh leaf material was homogenized in a mortar and pestle with a small quantity of acid-washed sand and 10 ml of 80% ethanol. The homogenate was filtered and the supernatant saved. The extraction was repeated twice with the residue. The volume of the residue was reduced by evaporation and the extract was used for the quantitative estimation of amino acids. Soluble amino acids were estimated according to the procedure of Lee and Takashi (1962) using ninhydrin reagent (0.1 g of ninhydrin was dissolved in 100 ml of water-saturated n-butanol). 1 ml of extract was made up to 2 ml with

distilled water. The tubes were heated for 20 minutes on hot water bath. After 15 minutes, the intensity of the purple colour was read against a reagent blank at 570 nm in UV-Visible spectrophotometer.

Estimation of Nucleic acid

Nucleic acids were extracted as per the method of Markham (1955). One g of leaf material was homogenized using ethanol. 5 ml of ethanol homogenate was hydrolyzed with 5 ml of 10% perchloric acid at 60^0C for 10 minutes. The suspension was centrifuged at 5,000 rpm for 10 minutes. The pellet containing protein was discarded. The supernatant was diluted to 10 ml with 0.5 N perchloric acid. This perchloric acid extract was used for the estimation of nucleic acids.

Estimation of DNA

Diphenylamine reagent

1.5 g of diphenylamine was dissolved in 100 ml of acetic acid, to which, 1.5 ml of concentrated H_2SO_4 was added and stored in a refrigerator in amber coloured bottle. 0.1 ml of 1:6 (v/v) aqueous acetaldehyde was added to 30 ml of previously prepared diphenylamine solution just before use. DNA content was estimated as per the method of Burton (1956). 2 ml of perchloric acid extract was diluted to 4 ml with 0.5 N perchloric acid. 4 ml of diphenylamine reagent was added to this and incubated at 30^0C for 17 hours. After the incubation period, absorbance of blue colour formed was measured at 600 nm in UV-Visible spectrophotometer. Simultaneously, a blank was conducted using only reagent and 0.5 N perchloric acid. The amount of DNA was calculated from a standard graph obtained by using known amount of DNA and expressed in milligrams/gram fresh weight.

Estimation of RNA

RNA content was estimated according to the method of Cherry (1962). 2 ml of RNA extract was diluted to 4 ml with 0.5 N perchloric acid. Then, the contents were heated with 4 ml of orcinol reagent (0.5 ml of $FeCl_3$ (10 % w/v) solution was diluted to 100 ml with concentrated HCl. 1g of orcinol was dissolved in the resulted solution and mixed thoroughly on a boiling water bath for 20 minutes. After cooling, the absorbance of the green colour was measured at 550 nm using the UV-Visible spectrophotometer. A standard curve was prepared with RNA (HiMedia).

Estimation of Indole Acetic Acid

One gram of leaf material was taken and homogenized with methanol. The homogenate was filtered and the filtrate was centrifuged at 7,000 rpm for 10 minutes in cold centrifuge. The supernatant was used for estimation of IAA. A 0.5 ml of supernatant was taken in test tubes and made up to 4 ml with distilled water. 4 ml of Salkowski reagent (1 ml of 0.5 M ferric chloride was mixed with 50 ml of 35% perchloric acid) was added to these test tubes and incubated for 30 minutes in the dark. After incubation period, the absorbance was measured at 530 nm with UV-Visible spectrophotometer. Amount of indole acetic acid was calculated with the help of standard graph. Amount of IAA was expressed in milligrams/gram fresh weight.

Estimation of Chlorophylls and Carotenoids

Chlorophylls and Carotenoids were estimated using the method given by Lichtenthaler (1987). 1.0 g of fresh leaf material was homogenized by using 100% acetone. Resulted green liquid was transferred to a Buchner funnel containing a pass of Whatman No.1 filter paper. The grinding was repeated till the tissue got devoid of any pigments with 100% acetone. Mortar and sides of the funnel were rinsed with 10 ml of 100% acetone to ensure that all the pigments are collected. Final volume was adjusted to 100 ml with acetone. The optical densities of pigment extracts were read at 661, 644 and 470 nm in spectrophotometer. The absorbance was measured against a 100% acetone solvent as blank. The pigment concentration was calculated using the following formula:

C_a = 11.24 A661 - 2.04 A644
C_b = 20.13 A644 - 4.19 A661
C_{a+b} = 7.05 A661 + 18.09 A644

$$C_{X+C} = \frac{1000 - 1.90\,C_a - 63.14\,C_b}{214}$$

Where,
C_a = Concentration of chlorophyll 'a' (mg/g fresh weight).
C_b = Concentration of chlorophyll 'b' (mg/g fresh weight).
C_{a+b} = Concentration of total chlorophylls (mg/g fresh weight).
C_{X+C}= Concentration of total carotenoids (xanthophylls and carotenes mg/g fresh weight).
A = Absorbance measurement at wavelength.

Estimation of Phenols

Phenol was estimated using the method given by Malick and Singh (1980). A 0.5 g of leaf material was homogenized with 80% ethanol. Supernatant was collected after centrifugation at 10,000 rpm for 10 minutes. Extraction was repeated twice to pool the supernatant. The supernatant was evaporated to dryness. The dried residue was dissolved in 5.0 ml of distilled water. From this, 0.1 ml was taken and the volume was made up to 3.0 ml using distilled water. To this, 0.5 ml of FCR was added and incubated for three minutes. After incubation, 2.0 ml of 20% Na_2CO_3 was added to each tube, mixed thoroughly and placed in boiling water bath for one minute. After cooling, the absorbance against a reagent blank was recorded at 650 nm. The amount of phenol present in test sample was calculated by using standard graph prepared from catechol.

Proline

Acid ninhydrin reagent

A 1.25 g of ninhydrin was mixed with 30 ml of glacial acetic acid and 20 ml of 6 M O-phosphoric acid and warmed with agitation until it dissolved. The reagent was stored at 4^0C and used within 24h. Proline was estimated using the method given by Bates *et al.* (1973). A 0.5 g of shoot material was taken in a cleaned pestle and mortar, homogenated with 5.0 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman No. 1 filter paper. The volume of the filtrate was made up to 10.0 ml by adding sulfosalicylic acid. 2.0 ml of filtrate was incubated along with 2.0 ml glacial acetic acid and 2.0 ml ninhydrin reagent and kept for boiling on water bath at 100^0C for 30 min. The reaction was terminated by placing the tubes on ice bath. 6.0 ml of toluene was added to the reaction mixture and stirred well for 20-30 sec. The separated toluene layer was pipette, warmed to room temperature and measured its red colour intensity at 570 nm against blank. The amount of proline present in the sample was calculated using the standard curve.

Hydrogen peroxide

Hydrogen peroxide was estimated using the method given by Velikova *et al*. (2000). Leaf tissue was homogenized in ice bath with 0.5 ml of 0.1%. (w/v) trichlroacetic acid. The homogenate was centrifuged at 10,000 rpm for 15 min. 0.5 ml of the supernatant was

added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1.0 ml of 1 M KI. The absorbance of the supernatant was measured at 390 nm.

Ascorbate

Ascorbate was estimated using the method given by Oser (1979). Leaf tissue was extracted with m-phosphoric acid. The reaction mixture consists of 2.0 ml of 2% Na-molybdate, 2.0 ml of 0.15 N H_2SO_4, 1.0 ml of 1.5 mM Na_2HPO_4 and 1.0 ml leaf extract were mixed and incubated at 60^0C in water bath for 40 min. Then cooled, centrifuged at 3,000 rpm for 10 min and absorbance was measured at 660 nm.

Glutathione

Glutathione was estimated using the method given by Griffith (1980). Leaf tissue was homogenized with 5% (w/v) sulfosalicylic acid and homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant (1.0 ml) was neutralized with 0.5 ml of 0.5 M potassium phosphate buffer (pH 7.5). Total glutathione was measured by adding 1.0 ml of neutralized supernatant to a standard solution mixture consisting of 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.5) containing EDTA, 0.2 ml of 6 mM NADPH and 1.0 ml of 1-U yeast-GR Type III. The change in absorbance at 412 nm was followed at 25 $\pm$ 2^0C until the absorbance reached 5U.

RESULTS

Biochemical analysis of plants grown in different composts Carbohydrate fractions

Effect of different compost on the content of carbohydrates in *C. roseus* plant was determined and presented in Fig 1. The starch content was maximum in plants grown in hair compost (18.22 mg) followed by feather (18.11 mg), mulberry (17.32 mg), cow dung (17.08 mg) and urea (17.0 mg), comparatively, treated plants showed more starch than control (16.34 mg). Total sugars content showed a slight difference in their amount among the treatments. The reducing sugars content was more in treated plants as compared to control. Maximum was in hair (0.68 mg) followed by feather (0.59 mg), mulberry (0.56 mg), cow dung (0.49 mg), urea (0.41 mg). Similarly, the non-reducing sugars were more in treated plants as compared to control.

Proteins

Effect of different compost on the protein content in the leaves of *C. roseus* was determined and presented in Fig 2. Maximum protein content was recorded in plants in hair compost (4.87mg/g), followed by feather (4.36 mg/g), mulberry (3.69 mg/g), cow dung (3.47 mg/g), and urea (3.17 mg/g). In control the protein content was more (3.29 mg/g) than the plants grown with urea.

Amino acids

Effect of different composts on amino acid contents in the levels of *C. roseus* was determined and presented in (Fig 3). Maximum amino acid content was recorded in plants grown in feather compost (6.1 mg) followed by hair (5.7 mg), mulberry (5.0 mg), cow dung (4.9 mg) and urea (4.12 mg). In control the amino acid content was more (4.55 mg) than the plants grown with urea.

Nucleic acids

Effect of different composts on nucleic acid content in *C. roseus* was determined and presented in (Fig 4). Maximum DNA content was observed in plants grown in hair compost (1.42 mg) followed by feather (1.41 mg), mulberry (1.21 mg), cow dung (1.08 mg) and urea (0.93 mg). RNA content was increased from 2.2 mg (in control) to 4.16 mg (in hair compost). Similarly, total nucleic acids are also increased from 3.112 mg (in control) to 5.58 mg (in hair). Hair composts showed more DNA, RNA and total nucleic acid content as compared to urea.

Indole Acetic Acid (IAA)

Effect of various composts on IAA content in *C. roseus* plant grown with various composts was determined and presented in Fig 5. IAA content was maximum in the plants grown with hair compost (1.2 µg) followed by feather (1.12 µg), mulberry (0.88 µg), cow dung (0.79 µg) and urea (0.69 µg). All the treated plants showed increase in IAA contents as compared to control (0.63 µg).

Chlorophyll and Carotenoids

Different composts were applied to the *C. roseus* plant and estimated total chlorophylls. Total chlorophylls were maximum in plant grown with hair (20.89 mg/g) compost followed by feather (20.18 mg/g), mulberry (18.7 mg/g), cow dung (11.62 mg/g) and urea (5.87 mg/g). The total chlorophylls in control (9.4 mg/g) plants were more than the plants grown with urea. Similarly, the carotenoid content was more in plants grown in hair compost (5.08 mg/g) followed by feather (5.03 mg/g), mulberry (4.22 mg/g), cow dung (3.37 mg/g) and urea (3.1 mg/g). The carotenoid content was also more in control (3.29 mg/g) plants the grown in urea. The phenol content varied from 4.22 mg/g to 2.52 mg/g in treated plants. Maximum phenol was estimated in the plants grown in hair compost (4.22 mg/g), followed by feather (4.14 mg/g), mulberry (3.28 mg/g), cow dung (3.10 mg/g) and urea (2.52 mg/g). The control (2.92 mg/g) plants showed more phenol than the plants grown in urea. The Proline content varied from 1.21 to 1.82. Maximum proline content was recorded in pants grown in mulberry compost (1.82 mg/g). This was followed by feather (1.61 mg/g), hair (1.58 mg/g), cow dung (1.12 mg/g) and urea (0.96 mg/g). The proline content in control (1.21 mg/g) plants was more than the plants grown in cow dung and urea as represented in Fig 6.

Hydrogen peroxide

Hydrogen peroxide estimated in the leaves of *C. roseus* grown in different composts was varied from 3.04 mg/g to 2.28 mg/g. Maximum hydrogen peroxide was recorded in plants grown in hair (3.04 mg/g), feather (2.82 mg/g), mulberry (2.28 mg/g). The H_2O_2 content of cow dung (2.02 mg/g) and urea compost (1.98 mg/g) decreases the H_2O_2 compared to the control (2.16 mg/g). The ascorbate content estimated it varies from 9.58 to 7.86 mg/g. Maximum ascorbate was recorded in the plants grown in feather compost (9.58 mg/g) followed by hair (9.47 mg/g), mulberry (8.70 mg/g), cow dung (8.16 mg/g) and urea (7.36 mg/g). The control plants showed more ascorbate (7.86 mg/g) than the plants grown in urea. Glutathione was estimated in leaves was gradually increased along with the cow dung (4.80 mg/g), mulberry (5.50 mg/g), feather (5.92 mg/g) and hair (6.18 mg/g) respectively as compared to glutathione in control (4.63 mg/g). However in urea compost treatment, the glutathione estimated was less (4.32 mg/g) than control as shown in the Fig 7.

DISSCUSSION

The Content of carbohydrate varies along with different composts. Gradual increase in starch from hair compost was observed. Total sugars content and reducing sugars shown maximum values in all composts when compared to control. In both cases maximum values increased in hair compost followed by feather compost. Decreased in

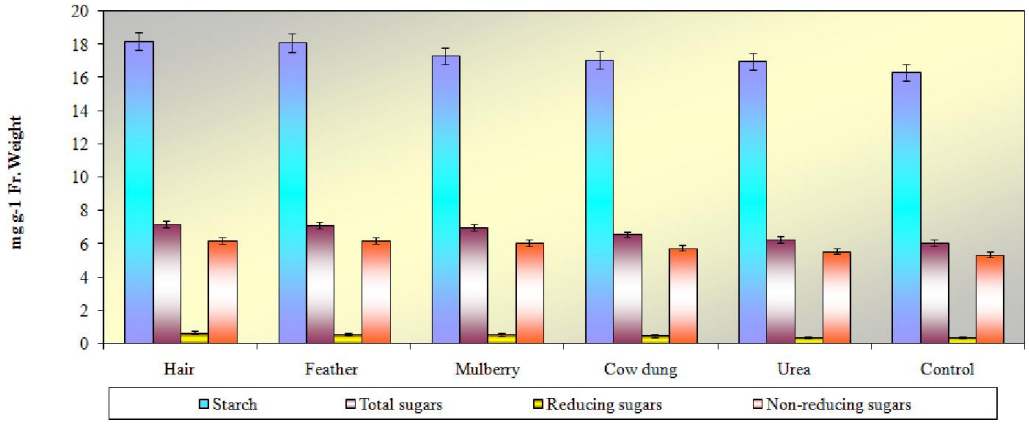

Fig. 1. The Effect of different composts on carbohydrate fractions in *C. Roseus*

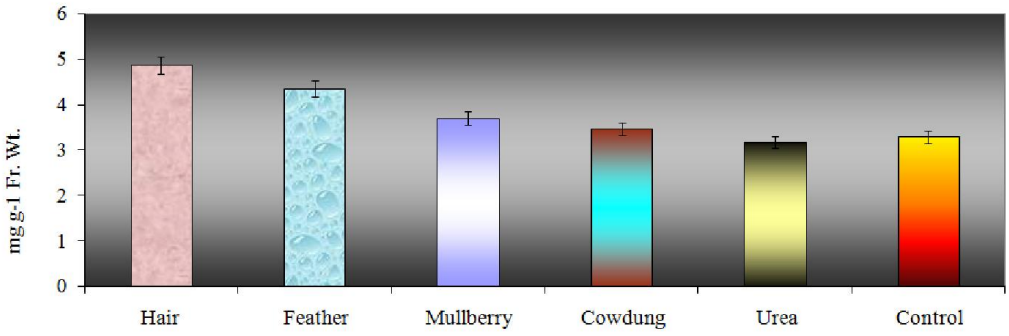

Fig 2. Effect of different compost on protein content in *C. Roseus*

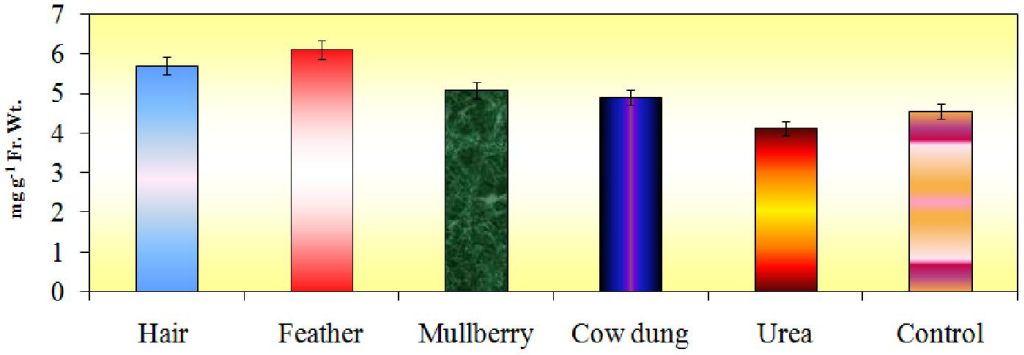

Fig. 3. Effect of different composts on amino acids contents in *C. Roseus*

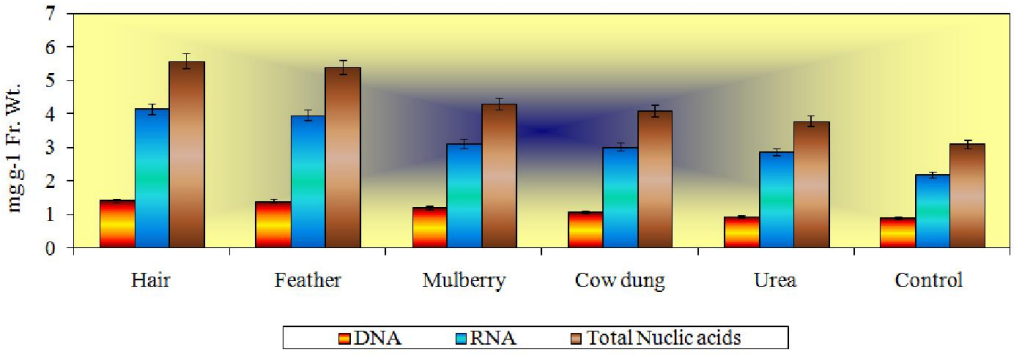

Fig. 4. Effect of different composts on nucleic acid contents in *C. Roseus*

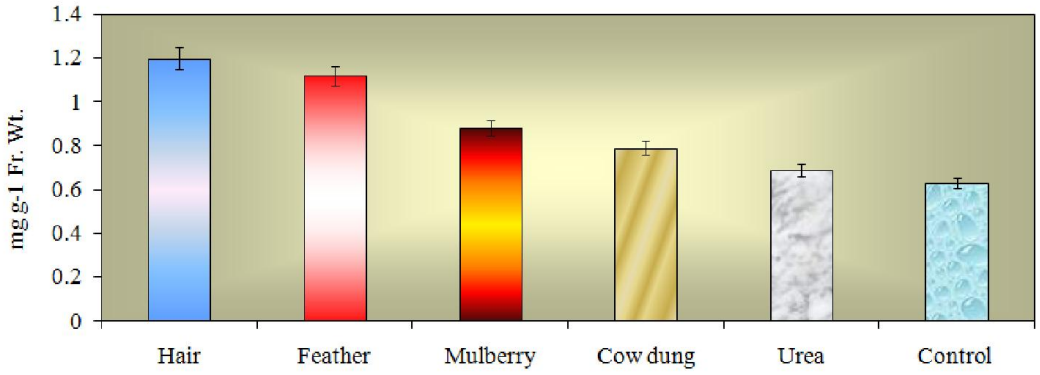

Fig 5. Effect of different composts on IAA content in *C. Roseus*

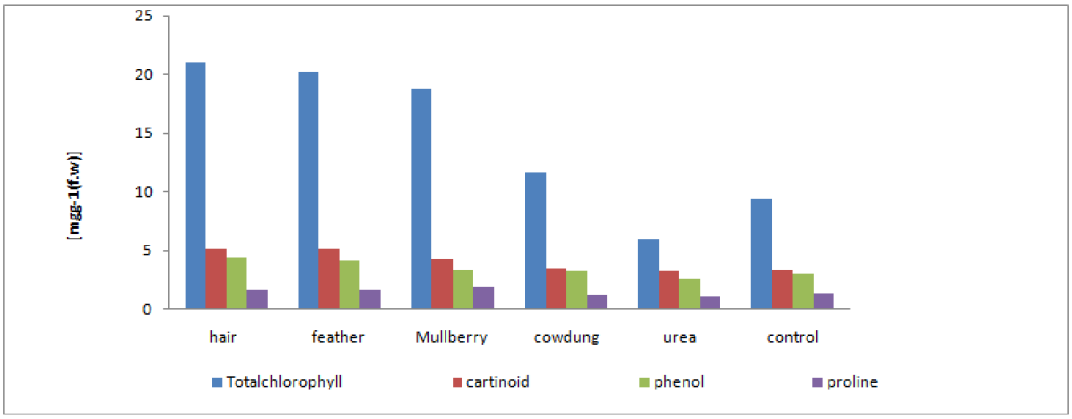

Fig. 6. Effect of different composts on the contents (A) Total chlorophyll (B) Carotinoid (C) Phenol (D) Proline

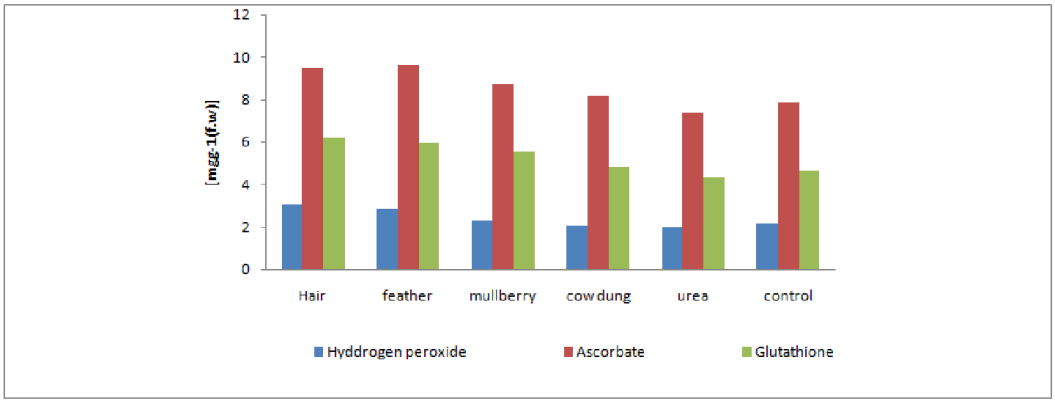

Fig 7. Effect of different composts on the contents (A) Hydrogen peroxide (B) Ascorbate (C) Glutathione activity of *C. roseus*

total sugars and non reducing sugars content and increased in reducing sugars content was well studied by Yang and Miller (1963) in soya bean leaves. The decrease of increase in the protein synthesis may be due to differences in the absorption of N_2 from soil, assimilation of absorbed nitrate or the translocation of organic nitrogen (Augustino, 1993). In the present study the protein content increased in hair as compared to control. In similar findings occurs sunflower and mung bean, the protein synthesis decreased as salinity increased (Sen Gupta *et al*., 1993) but, it was found to be increased in barley (Singh and Tewari, 2003). Increase in the concentration of amino acids was reported in fluoride treated soyabean leaves by Yang and Miller (1963). In the present study, amino acid level was increased in feather compost and decreased in urea as compared to control. The changes in free amino acid pool depend upon degradation of storage proteins, amino acid synthesis and utilization of amino acids for protein synthesis and respiration. Total nucleic acid content was increased in different composts applied to *C. roseus* plant. DNA and nucleic acid content also increased from hair compost

as comparing to all other composts. Similar results observed by decrease in DNA and RNA content was reported by Narita *et al*. (1996) and Chang and Thompson (1996). Reduction in RNA and increase in RNase activity in lentil roots were reported by Pilet (1969). In the present study IAA content increased from the hair and feather compost and urea fertilizer as minimum as compared to control one. Increases the IAA there are many internal and external factors that affect the overall growth and development of plants. One such internal factor is plant hormone, which helps in transmitting signal between the cells and within the cells. Previous findings have conjectured that endogenous content of plant hormones such as abscisic acid, auxin, cytokinins, zeatin and gibberellins changes in response to salt stress (Javid *et al*., 2011). In the present study, IAA was estimated to explore the effect of composts on plant hormones. Total chlorophyll and carotenoid content increased in the leaves of compost treated plants, except urea. Break down of chlorophyll and concomitant enhancements in carotenoids are well established components of senescence (Thomas and Stoddart, 1980).

It is well documented that carotenoids are involved in the protection of photosynthetic apparatus against photoinhibitory damage by single oxyzen (O^{-2}), which is produced by the excited triple state of chlorophyll (Siefermann- Harms, 1987; Foyer and Harbinson, 1994). The Phenolic compounds, a diverse group of plant secondary metabolites, play an important role in the regulation of plant growth, development including germination and early seedling growth (Howell and Kremer, 1973). In the present study, phenol content was increased significantly in treatment with different composts over control except urea. Proline acts as a hydrophobic protectant for enzymes and subcellular organelles (Lerudulier *et al*., 1994). In the present study, proline content was varied in different type of compost treatment on plants. Mulberry and feather composts showed increase in proline content as compared to cow dung, hair and urea compost and control. An increase in proline content may serve as means of protection of plant tissue, helps in maintaining water relations, leading to serious damage in cellular components and DNA lesions. The Hydrogen peroxide is produced mainly in the photorespiration glycolate pathway in fatty acid β-oxidation, in the enzymatic reaction of flavin oxidases and in the disproportion of O^{-2} radicals to H_2O_2 (Hurng *et al*., 1983; Del Rio *et al*., 1996) and higher concentration is injurious to cell/plant resulting in lipid peroxidation and membrane injury (Baisak *et al.,* 1994; Menconi *et al*., 1995). The present study reveals an increase in H_2O_2 content in the plants grown with feather, hair and mulberry composts as compared to cow dung and urea compost and control. The role of ascorbate has been reported in the thylakoid surface within the chloroplast, where, it acts as reductant in the APX-mediated scavenging of H_2O_2 (Grace *et al.,* 1995). Ascorbate also acts as a reductant in the regeneration of a-tocoperol and in the Zeaxanthin cycle (Foyer, 1993). In the present study, the amount of ascorbate was significantly more in plants grown with feather, hair, mulberry and cow dung composts as compared to control. Urea compost had shown decreased content of ascorbate. The amount of glutathione estimated in *C. roseus* leaves was gradually increased in an order of cow dung (4.80 mg/g), mulberry (5.50 mg/g), feather (5.92 mg/g) and hair (6.18 mg/g) and in control the amount was 4.46 mg/g. The plants grown with urea showed less glutathione than control. Glutathione is a major low molecular weight the iso-compound present in most plants (Foyer *et al*., 2001). A great reduction in glutathione (GSH) content in different parts of *C. roseus* plants under different concentrations of NaCl treatments (Jallel *et al*., 2006). GSH also plays a protective role in salinity tolerance by the maintenance of the redox status (Shalata *et al*., 2001). GSH synthesis has been shown to respond either directly or indirectly to hydrogen peroxide (Smith 1985).

Conclusion

These results demonstrate that hair and feather compost was more effective than other compost in *catharanthus roseus* plant. Plants grown in soil mixed with these composts compared to the control. There were significant interaction effects hair and feather compost between debris of mulberry leaves compost, cow dung and urea fertilizer effects on studied biochemical characteristics of periwinkle. This can be attributed to improved waste keratinous composts in agricultural soil fields in favor of plant growth and development particularly by increasing the activity and more and nutrients uptake, more vegetative growth. It is also possible that some hormones are produced to help plants tolerate environmental stress. Application of hair and feather composts improves soil fertility along with other soil properties and could be an attractive natural fertilizer for crops. This method could be an alternative to farm composting to produce a community oriented and eco-friendly compost.

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