INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

EFFECT OF FLY ASH ON SOME BIOCHEMICAL PROPERTIES OF *VIGNA MUNGO* L.

KULDEEP KUMAR, ASHOK KUMAR

Department Of Botany, C. C. S. University, Campus Meerut-250004

Accepted Date: 22/12/2016; Published Date: 27/04/2017

Abstract: Study was conducted on the effect of fly ash on the biochemical properties of *Vigna mungo* L. Results obtained reveal that the increasing proportion of fly ash in soil and plants considerably increases the value and certain characteristics. Effect of these changes show positive changes on protein, nitrogen, proline, leghaemoglobin, chlorophyll, and other biochemical properties of *Vigna mungo* L. All these were found to be favourably affected by fly ash induced changes in biochemical characters of the plant. Hence, through the present study one can conclude that fly ash in moderate percentage can be a boon for sustainable agriculture.

Keywords: Fly Ash, *Vigna mungo L.*, Biochemical Properties

PAPER-QR CODE

Corresponding Author: MR. KULDEEP KUMAR

Access Online On:

www.ijprbs.com

How to Cite This Article:

Kuldeep Kumar, IJPRBS, 2017; Volume 6(2): 1-13

INTRODUCTION

Legumes are considered to be a very important group of plant food stuffs, particularly in the developing world, as a cheap source of protein when animal protein is scarce. A significant part of human population relies on legumes as staple food for subsistence, particularly in combination with cereals. They are unique foods because of their rich nutrient contents including starch, protein, dietary fibre, oligosaccharides, photochemical and minerals (Borade *et al.* 1984). India produces about 1.5 million tons of urd annually from about 2.5 million hectares of area with an average productivity of 400 kg/ hectare. It contains perfect combination of all nutrients, which includes proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins (Karamany, 2006).

The study of Singh *et al*. (2014) found on fly ash clearly shows that its application as an amendment to agricultural soil can significantly improve soil quality as higher soil fertility, which is known to be essential for the yield of legumes to fulfil the requirement of pulses for the growing population of the country.

Agriculture and waste land management have emerged as prime bulk utilization areas for fly ash in the country. It improves permeability status of soil; improves fertility status of soil (soil health); crop yield; improves soil texture; reduces bulk density of soil; improves water holding capacity and porosity; optimizes pH value; improves soil aeration; reduces crust formation providing micro-nutrients like Fe, Zn, Cu, Mo, B, Mn; provides macro nutrients like K, P, Ca, Mg, S, etc; works as a part substitute of gypsum for reclamation of saline alkali soil and lime. For exclamation of acidic soils, ash ponds provide suitable conditions and essential nutrients for plant growth, help to improve the economic condition of local inhabitants; crops grown on fly ash amended soils are safe for human consumption without affecting groundwater quality. Use of fly ash in agriculture has also proved to be economically rewarding (Katait, 2016).

The present investigation, the effect of fly ash on *Vigna mungo* L. was undertaken with a view to develop data from field experiments on the beneficial effects of fly ash with special emphasis on changes in biochemical properties of the plant.

MATERIAL AND METHODS:

The present investigation was conducted to find out the “Effect of fly ash on Physio-chemical properties of soil grown *Vigna mungo* L.”. The details of material used for experimental purposes and techniques adopted in the present investigation are described as follows.

Geographical Situation: Meerut district is situated between 29^0 01N latitude and 77^0 45E longitude at an altitude of 237 meters above sea level. The C.C.S. University is situated at the distance of about 10km from Meerut city railway station and near about 12km on Delhi-

Dehradun highway. The total geographical area of Meerut district is 2564 km^2. The district falls under western plain zone of Uttar Pradesh, sub region of upper Gangetic plain.

Experimental site: The field experiments were conducted during the kharif season in the month of March to June in 2016 to evaluate the response of Fly ash on the Physio-chemical properties of soil and growth and yield of *Vigna mungo* L. The seeds of *Vigna mungo* L. were grown in the field of Botany Department, C.C.S. University, Meerut. The experiment was designed in four plots of equal size (1×1meter), three plots for the treatment and one plot for the control. Three samples of different amount of fly ash were prepared such as 25g, 50g, and 100g. First plot was designed for control; second plot was treated with 25g, third plot with 50g and fourth plot with 100g of Fly ash.

For experimental work, 1kg soil taken from of the each plots and fly ash such as 25g, 50g and 100g was mixed evenly, and then it is distributed in each of the plot uniformly except in the control before sowing of *Vigna mungo* L. seeds. Fly ashes of different amounts were mixed with soil as following:

1. 25g X 1kg
2. 50g X 1kg
3. 100g X 1kg

Soil was treated with fly ash according to the above method. Then treated soil with Fly ash is spread in each of three plots, and then mixed soil is uniformly distributed in the three plots.

Material used:

1. Certified seeds of *Vigna mungo* L. were collected from IARI, New Delhi.
2. Fly ash was collected from the fields near brick kiln at Hastinapur Meerut, U.P. INDIA.

Other Details (Experimental Details);

1. Total no. of block – 4
2. Control - 1
3. Total no. of treated plots – 3
4. Plot size (area of plot) – 1X1 meter.

Forty healthy seeds of *Vigna mungo* L. were sown in every plot. All plots were irrigated with tap water. The seed germination percentage was calculated after counting the difference between germinated (coming out of soil) and non-germinated seeds (remaining inside, non emergent).

Figure: A *Vigna mungo* L. (Control)

Figure: B *Vigna mungo* L. (Treatment-I)

Figure C: *Vigna mungo* L. (Treatment-II)

Figure D: *Vigna mungo* L. (Treatment-III).

Determination of Protein:

The protein was estimated by the method adopted by Bradford (1976). To determine the protein contents in the nodules, the plants were uprooted carefully from the soil and washed thoroughly to remove soil particles. The nodules were separated from roots and blotted to remove the water content from them. After proper blotting the nodules were weighed (100mg) and crushed of pre-chilled mortar-pestle with ice mixture in cold homogenizing Tris-EDTA buffer. The slurry was centrifuged at 5^0C at 10,000 rpm for 10 minutes to get clear supernatant. The supernatant was used as nodular protein extract for the estimation of total nodular protein and leghaemoglobin. In the supernatant, total soluble nodular proteins were estimated according to the dye binding method of Bradford (1976). Supernatant was divided in three replicates in the test tube. 1ml supernatant in each test tube and 5ml brilliant blue added in

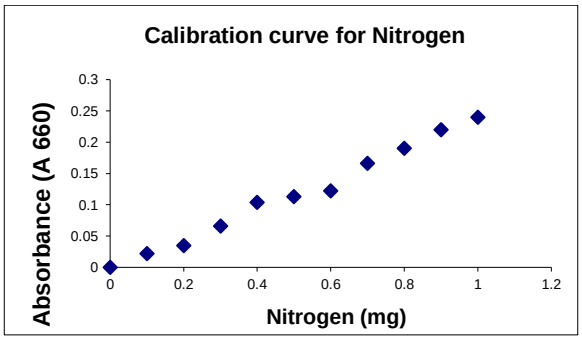
each test tube. Incubates the test tube (whole solution) approximately for 15-20 minute at room temperature, and then take O.D. at A_{595} nm. In this way total soluble nodular protein was estimated. Casein was used for the preparation of standard curve for the estimation of nodular protein. In case of blank only buffer equals to the amount of dye was taken and mixed several times by gentle shaking of the test tube. After the standing period for 5-15 minutes, the colour developed in the supernatant. Then O.D. was taken at A_{595} nm for the various nodular samples. Protein concentration of the samples was calculated as mg/gm fresh weight (Bradford, 1976).

The following formula was used for the measurement of protein content:

O.D. x Factor x Dilution (if any) x 1000/100 x Total volume/volume of replicate.

Estimation of Total Nitrogen:

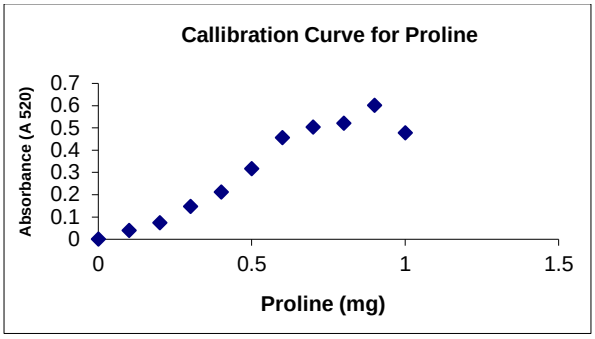
Total nitrogen was estimated by Snell and Snell, (1967) method. For this 50 mg of sample (nodule) was taken and digested with appropriate amount of digestion mixture (5.0 ml of conc. H_2SO_4 and 2.0 ml of 30% H_2O_2). The test tubes were kept on hot plate (sand bath) for 30 min. for digestion, after that test tubes were further cooled at room temperature and again added with 3.0 ml of 30% H_2O_2 and kept for further digestion for 60 min. or till the digest became clear. Make total volume 10 ml with double distilled water. After taking 1.0 ml of digest and added with 3.0 ml of Nessler's reagent and 1.0 ml of distilled water. (3.0 ml Nessler's reagent + 2.0 ml of DDH_2O), the total Nitrogen was calculated using formula given by Koch and McMeak in and developed the colour was further recorded at A_{425}. For the standard curve of Nitrogen a standard solution of 0.5mM $(NH_4)_2SO_4$ was prepared. From this solution, 1.0 ml solution was added to the test tube containing 3.0 ml Nessler's reagent and finally the volume was made up to 5.0 ml with DDH_2O. Absorbance was taken against a blank (3.0 ml Nessler's reagent + 2.0 ml DDH_2O) at A_{425} (Snell and Snell, 1967).



Estimation of Proline content:

Total proline contents of nodules was estimated by the following protocol given by Bates *et al*. (1973, 1952). For the experiment 100mg of plant material was homogenized in 10ml of 3%

aqueous sulphosalicylic acid. The homogenate was filtered with Whatman No. 2 filter paper. After this 2ml of filtrate was taken in a test tube and 2ml of glacial acetic acid was added along with 2ml of acid ninhydrin solution. The sample was further placed in the boiling water bath for heating for about 1hfor termination of the reaction. Further 4ml toluene was added to the reaction mixture and stirred well. Then the toluene layer was separated. The absorbance was taken at 520 nm. A series of standard with pure proline was run in a similar way and got prepare a standard curve (Bates *et al.,* 1973).



Leghaemoglobin content analysis:

Leghaemoglobin quantities of the nodules were measured spectrophotometrically as hemochromogen according to the method of Bergersen (1980).To determine the leghaemoglobin content in the nodules mix fresh or thawed nodules with 1-3 volume of phosphate buffer (100mg nodules + 200µl phosphate buffer) and macerate in mixer filter through two layers of filter paper. Nodule debris was discarded and remainder reddish brown filtrate was centrifuge at 10,000 rpm for 10-30 minutes and further diluted. To an equal volume, alkaline pyridine reagent was added and mixed (2-5ml). The solution becomes greenish-yellow due to the formation of ferric hemochrome. The hemochrome was equally divided into two test tubes. In one portion add few crystals of sodium dithionite to reduce the hemochrome and stirred without aeration. To the other portion, add few crystals of potassium hexacyanoferrate for oxidize hemochrome and the contents of both the test tubes were measured at A556 nm. Then calculated the leghaemoglobin content by using the following formula,

$$\text{LB: Protein} = \frac{\text{LB/g fresh weight of nodule X 100}}{\text{Protein/g/fresh weight of nodules}}$$

Estimation of Chlorophyll content:

Chlorophyll content was estimated by using Arnon's (1949) method. For that 50 mg fresh leaf material was homogenized in 5.0 ml of chilled acetone (20 ml DW +80 ml acetone using a pinch of Na_2CO_3 in medium). The extract was centrifuged at 5000 rpm for 5 min. and the supernatant

was collected. The final volume was made after adding 5.0 ml of distilled water and absorbance was read at 645 and 663 nm against 80% acetone as blank(Arnon, 1949). For calculation the following formula is used:-

$$\text{Chl. a (mg/g f wt)} = \frac{12.7(A663) - 2.69(645)X\ V}{1000X\ W}$$

$$\text{Chl. b (mg/g f wt)} = \frac{22.9(A645) - 4.89(A663)xV}{1000xW}$$

$$\text{Total Chl. (mg/g f wt} = \frac{20.2(A645) - 8.02(A663)x\ V}{1000xW}$$

$$\text{Carotenoid} = \frac{7.6(A445) - 8.02(A663)x\ V}{1000x\ W}$$

Where,

V= Final volume of chlorophyll extract

A= Absorbance at specific wavelength

W= Fresh weight of tissue extract

Polypeptide pattern through Gel electrophoresis:

Take 300 mg nodules sample and crushed in 1.0 ml of extraction buffer (100mM Tris-Buffer 8.0 or 8.5pH). The extract was centrifuged at 12000 rpm at 4ºC for 10 minutes and the supernatant was collected. To the supernatant 100 µl protease inhibitor solution and 20 µl 50 mM NaCl solution were added and 20 µl supernatant aliquot was mixed with 10 µl 4X protein loading buffer (0.5 M tris pH 6.8-5.0 ml, SDS 0.8 g, Glycerol-20 ml, â- mercaptoethanol-2.0 ml, Bromo phenol Blue-1 or 2 Crystal, Water – to make final volume 10.0 ml) boiled for 5 minutes. 20 µl samples were loaded in gel. 10% (as standardized) poly- acrylamide SDS gel was used for electrophoresis run and in all the cases 45-55 µg protein (equivalent µl of protein extracted) were loaded (as the quantitative estimation of each sample was also done following Bradford *et al*., 1976 method. Gel was stained with Coomassie Brillant Blue (CBB R250-0.62g methanol-200 ml, Acetic acid (glacial) - 25 ml making total volume with water 500 ml) and de-stained with 10% acetic acid obtained for visual observations (Laemmli, 1970). Finally gels were scanned by gel documentation system and analyzed with the help of Biovis software.

RESULTS AND DISCUSSION:

Protein: It was noticed that addition of low amount of fly ash to the soil increases the protein contents as compared to control. However, high level of fly ash was found to reduce the protein content. This result shows that low amount of fly ash exhibits a positive effect on protein content. In 2015Pani, N.K.*et al*. reported that protein content significantly increased with lower (25%) fly ash application. However, reduction in the protein content was also reported under higher level of fly ash. Decline in protein content may result from an increase in the activity of protease or other catabolic enzyme. Such types of results have also been quoted earlier by Sharma *et al.* (2010) working on *Pisum sativum* L. and by Gautam *et al*. (2012) in *Brassica juncea* (Fig-1).

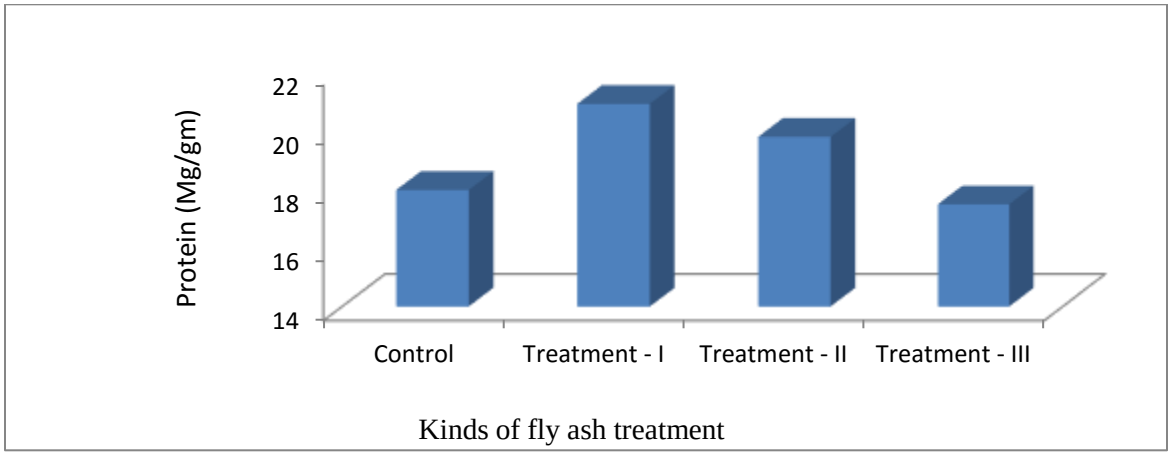


Figure 1: Effect of fly ash on protein content of *Vigna mungo* L.

Nitrogen: It was noticed that application of fly ash in lower amount increase the level of nitrogen content. Hence forth fly ash reduces the protein contents of plants as also observed during the present experiment. It could be due to the presence of arsenic in fly ash. The presence of Arsenic in fly ash shows the symptoms of phytotoxicity and considerable inhibition of initial growth of young black gram plants (Sarangi and Mishra, 1998). This is the reason of a decrease in N_2 content with an increase in the concentration of fly ash (Fig-2).

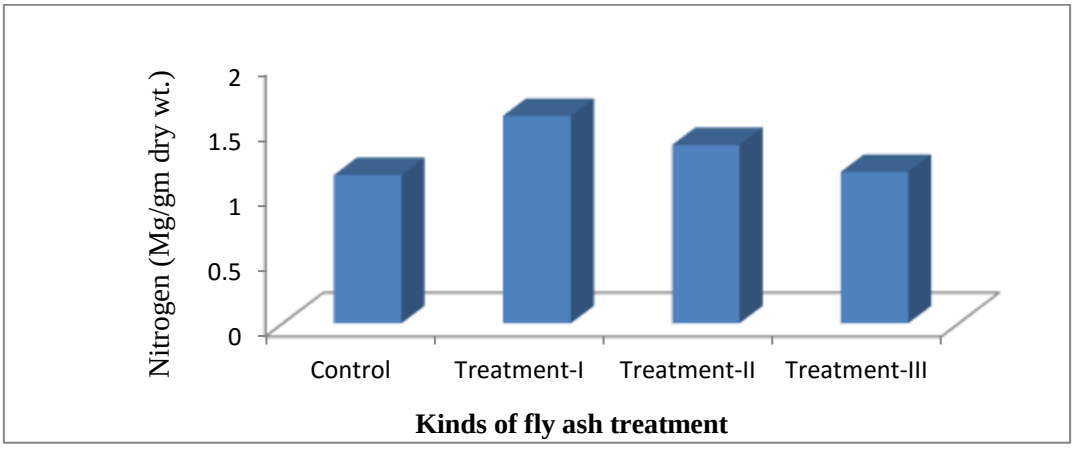


Figure 2: Effect of fly ash on Nitrogen content of *Vigna mungo* L

Proline: Increased level of proline contents in treatment-III indicates that fly ash induces drought stress to plants with an enhancement of proline contents. As reported by Page *et al.* (1979) fly ash contains Ni and Pb (heavy metals). Plants treated with grown in the presence of Pb & Ni contain by fly ash are responsible for the accumulation of proline contents in treated plants Singh *et al.* (2012) (Fig-3).

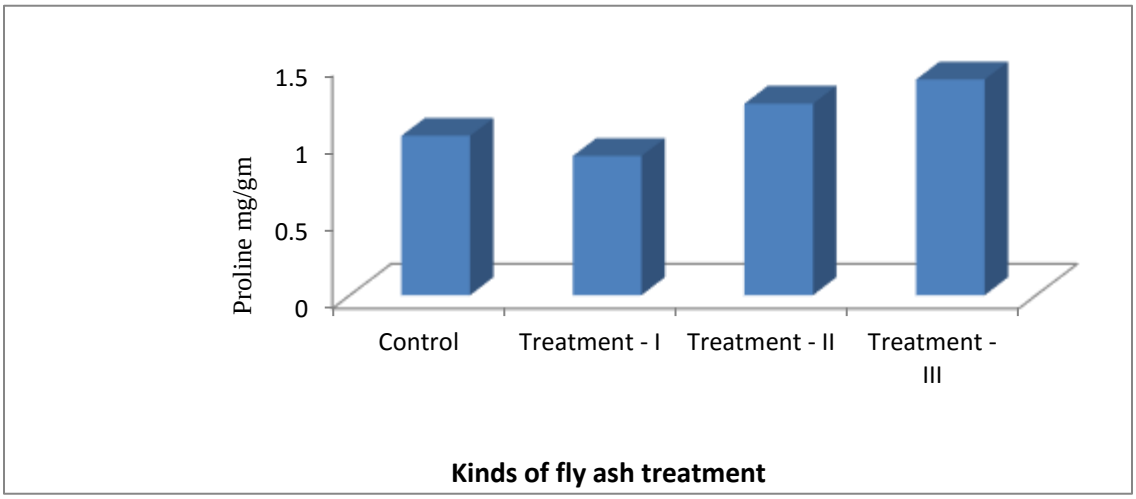


Figure 3: Effect of fly ash on proline content of *Vigna mungo* L.

Leghaemoglobin: Applications of fly ash were found to increase the level of leghaemoglobin as compared to control. This may be due to an increase in the porosity of soil that lead to high availability of oxygen around root system of plants that increase the level of leghaemoglobin is responsible for induction of anaerobic condition for nitrogenase enzyme so that plants can fix more nitrogen contents. Das *et al.* (2000) reported a significant increase in leghaemoglobin formation, when Co is applied; this played a role of an essential component of Nitrogen

fixation. Enhancement in the nodule number per plant ultimately increased the yield of soybean (Fig-4).

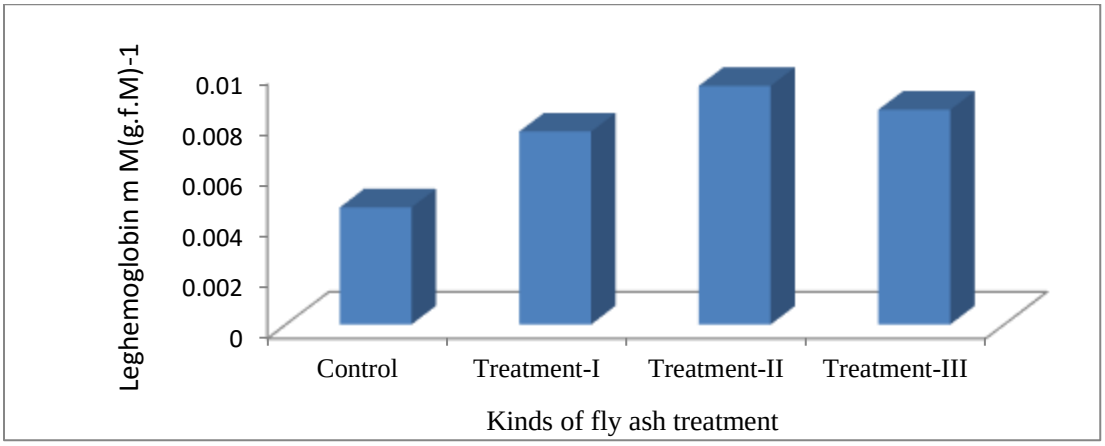


Figure 4: Effect of fly ash on Leghaemoglobin content of *Vigna mungo* L.

Chlorophyll: Addition of low amount of fly ash in the present experimentation was found to increase the total chlorophyll contents while reverse held true with the use of high amount of fly ash. The presence of lead result in reduction of the chlorophyll content in *Vigna mungo* L. has also been worked out by Singh and Siddhiqui (2003). Gluscote (1997) reported fly ash is good source of trace element for chlorophyll formation (Fig-5).

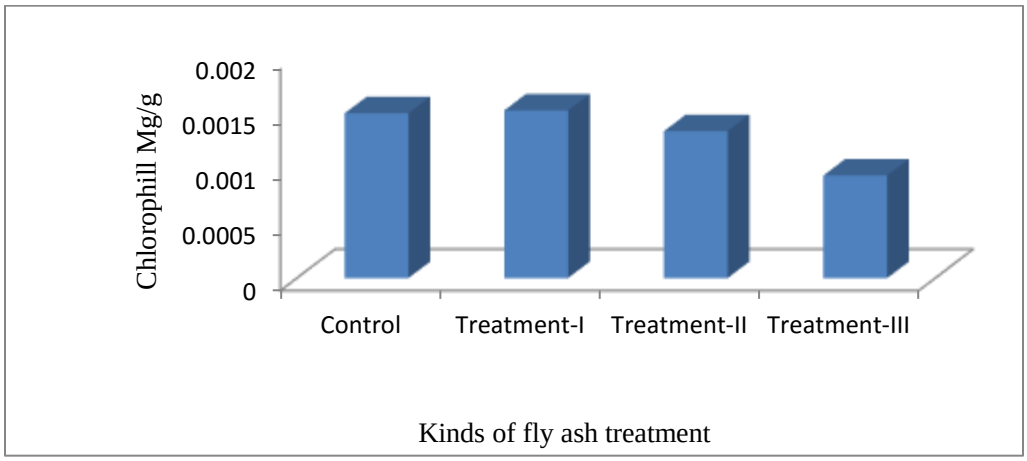
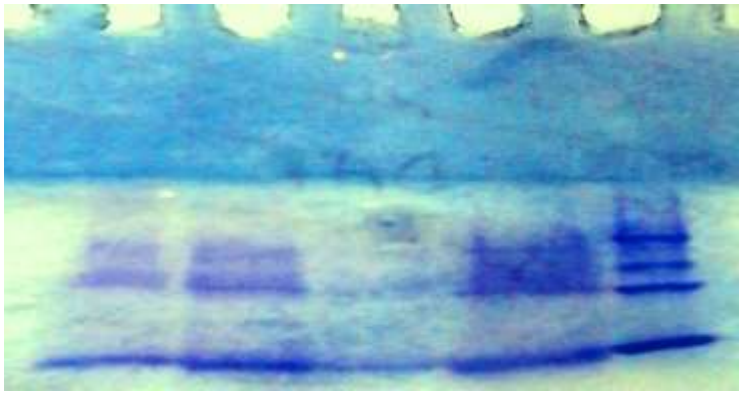


Figure 5: Effect of fly ash on chlorophyll content of *Vigna mungo* L.

Polypeptide pattern:

During protein profiling same banding patterns are observed in control and in all treated samples beside marker were also loaded in the first row of the gel (Fig-6).



5 4 3 2 1

Where,

1- Standard marker

2- Control

3- Treatment-I

4- Treatment-II

5- Treatment-III

Figure 6: Peptide pattern of proteins of *Vigna mungo* L.

CONCLUSION:

After analysing all the results of experiment it was concluded that application of fly ash to a certain limit results in an increase of available macronutrients and micronutrient of the soil. The biochemical parameters were significantly increase except proline in treatment-I. Fly ash acts as an excellent soil modifier and conditioner a very good liming agent, and a source of essential plant nutrients for appreciably improving the texture and fertility of the soil with significant increases in crop yield over the control. Fly ash in low concentration may serve as an opportunity to be used as an eco-friendly non-conventional fertilizer. At the same time it will be safe and eco-friendly disposal option for huge amount of fly ash. However, there is a need of detail study to declare fly ash totally safe and eco-friendly to be used as for fertilizer.

REFERENCES:

1. Arnon DI: Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. Plant physiology 1949; 24: 1-15.

2. Bates L S, Waldren R P and Tear I D: Rapid determination of free proline for water stress studies. Plant and soil. 1973; 39: 205-207.
3. Bergersen F J, Turner G L: Journal of General Microbiology. 1980; 118: 235-52.
4. Borade, V P, Kadam S S and Salunkhe D K: Changes in phytate, phosphorus and minerals during germination and cooking of horse gram and moth bean. Qual. Plant. Plant Foods for Human Nutrition 1984; 34:151-156.
5. Das A K, Uhler M D, Hajra A K: Molecular cloning and expression of mammalian peroxisomal trans-2-enoyl-enzyme A reductase cDNAs. Journal of Biochemistry. 2000; 275: 24333-24340.
6. El-Karamay M F: Double purpose (Forage and seed) of mung bean production -effect of plant density and forage cutting date on forage and seed yields of mungbean (Vigna radiata L. (Wilczck). Research journal of agriculture and biological sciences. 2006; 2: 162 – 165.
7. Gautam S, Singh A, Singh J and Shikha: Effect of Fly ash amended soil on growth and yield of Indian mustard (Brassica junicea). Advance in Bioresearch. 2012; 3 (4): 39-45.
8. Gluscote H.J.: Trace elements in Coal Ciro 499 lllinots State U.S. 1997; pp. 154.
9. Katait K S: Fly ash in India: From waste pollutant to eco-friendly Resorces, International Journal in Management and Social Science. 2016; 04 Issue-03: 471-481.
10. Laemmli U K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227(5259): 680-685.
11. Marion M. Breadford: A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry. 1976; 72: 248-254.
12. Page A L, Elseewi A A and Straughan I R: Physical and Chemical properties of fly ash from coal-fired power plants with special reference to environmental impacts. Residue Reviews Journal. 1979; 71: 83-120.
13. Pani N K, Samal, P, Das R, Sahoo, S L: Effect of fly ash on soil properties, Change in biochemical parameter and heavy metal uptake in sunflower (Helianthus annuus L.). International Journal of Science Technology and Management. 2015; Vol. 4: Issue 07.
14. Sarangi P K, Mishra P G: Soil metabolic activities and yield in ground nut in fly ash amended soil. Research Journal of Chemistry and Environment. 1998; 2 (2): 7 – 13.
15. Sharma S, Sharma P, and Mehrotra P: Bioaccumulation of Heavy Metals in Pisum sativum L. Growing in Fly Ash Amended Soil. Journal of American Science. 2010; 6(6): 43-50.
16. Singh L P, Siddhiqui A: Effect of fly ash on growth and yield three cultivars of rice. Bio resource Technology. 2003; 86 (1): 73 -78.
17. Singh G, Agnihotri R K, Sharma R, Reshma and Mushtaq Ahmad: Effect of lead and nickel toxicity on chlorophyll and proline content of Urd (Vigna mungo L.) seedlings. International Journal of Plant Physiology and Biochemistry. 2012; 4(6): 136-141.

18. Singh R P, Sharma B, Sarkar A, Sengupta S, Singh P, Ibrahim M H: Biological Responses of Agricultural Soils to Fly-Ash Amendment. Reviews of Environmental Contamination and Toxicology. 2014; 232**:** 45-60.
19. Snell F D and C T Snell: Colorimetric method of analysis including photometric methods Van Nostrand, Inc; Princerton, New Jersey 1967; 4: 217 D.