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POTENTIAL USE OF BRICK KILN COAL FLY ASH TO AMELIORATE BIOCHEMICAL PARAMETERS AND NITROGEN FIXATION EFFIECIENCY OF *PISUM SATIVUM* L.

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Abstract: Fly ash is a waste by product of coal combustion. It such as recovered from the gases of burning coal from brick kiln and thermal power plants and other industries which use coal for production of energy. Fly ash contains different types of nutrients such as Ca, S, Cu, Zn, Mg, P, Mn, B, and Fe, which are remunerative for plant growth. The continuous use of chemical fertilizers increases the crop productivity but also degrades physical and chemical quality of soil. The present study was conducted to detect the impact of different levels (25 gm, 50 gm, and 100 gm per meter^2) of fly ash on the determination of physicochemical properties of plant *Pisum sativum* such as protein, proline, nitrogen, leghaemoglobin, phosphorous, and some nitrogen fixing enzyme of *Pisum sativum.* All these were found to be favorably affected by fly ash induced changes in physicochemical properties of *Pisum sativum* L. Hence, through the present research work can conclude that fly ash in optimize amount (25gm/m^2) can be a mileage for sustainable agriculture.

Keywords: Brick kiln, crop productivity, Fly ash, fertilizers, nitrogen fixing enzyme.

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INTRODUCTION

Legumes are valuable source of proteins for the human diet. There are more than 13,000 species of legumes, but only 20 are eaten by mankind. The most consumed varieties of legumes are *Lens esculenta, Cicer arietinum, Vicia faba, Pisum sativum* and beans (Karmasan Harris, 1988). The nutritional contents of legumes can provides a high percentage of proteins, fats, carbohydrates, dietary fibers, B-complex vitamins (thiamin, riboflavin, niacin), and minerals as reported by Prodanov *et al.* (2004).

Pea (*Pisum sativum* L.) is a cool season food legume crop. It (*Pisum sativum* L.) belongs to the legume family (Fabaceae).Two types of pea can be distinguished: garden pea (green pea) and field pea (dry pea). It is an important crop due to their high iron, starch and protein contents (Dahl *et al.,* 2012). It is also one of the most important vegetables in the world and its ranks among the top 10 vegetable crops. It is mainly used in human diet throughout the world. It is a rich source of protein (21-25 %), carbohydrates, vitamin A and C, calcium, phosphorous etc. Pea seeds contain high levels of amino acids such as lysine and tryptophan as reported by Bhat *et al*. (2013). Like other legumes crop pea crop cultivation also maintains the soil fertility through the biological nitrogen fixation in association with symbiotic relationship of the *Rhizobium* prevalent in its root nodules. It also plays a vital role in cropping, fostering and in sustainable agriculture (Negi *et al.,* 2006).The long term uses of inorganic fertilizers has been implicated in soil acidification, loss of organic carbon, nutrient imbalance and deficiency of macro- and micro nutrients. Many studies introduced that fly ash can be used as a soil ameliorate that may enhance the physical, chemical and biological properties of the degraded soils (Kumar, 2017).

Application of fly ash in agricultural sector supports in replacing of chemical fertilizers. Crop plants of the families Leguminoceae, Brassicaceae, Chenopodiaceae and Poaceae are most tolerant to fly ash (Cheung 2000). Fly ash has although been identified as pollutant and fertilizer both, but the work done earlier was confined to some physico-chemical attributes. Present work is aimed at exploring the fertilizer potential of kiln coal fly ash in terms of nitrogen fixation efficiency modulation. The significance of legumes as food plants and fly ash as one of the pollutants in soil and air is also well known. The purpose of the present study was to convert a pollutant into a fertilizer.

MATERIAL AND METHODS

Experimental site: The field experiments were conducted during the Rabi season in the month of November to January during 2017-2018 to evaluate the response of fly ash on the biochemical property of *Pisum sativum* L. The seeds of *Pisum sativum* L. were grown in the field of Botany department, C.C.S. University campus, Meerut (U.P.). The experiment designed in four plots of equal size (1×1meter^2), three plots for the treatment and one plot for the control. Three samples of different concentrations of fly ash were prepared such as 25 gm, 50 gm, and 100 gm per meter^2. First plot was untreated and the remaining three plots were designed for 25, 50 and 100 gm/m^2 fly ash treatments.

MATERIAL USED

1. Certified seeds of *Pisum sativum* L. were collected from IARI, New Delhi.
2. Fly ash was collected from the brick kiln at the town Hastinapur Meerut.

RECORDED PARAMETERS

Chlorophyll content: Chlorophyll content was estimated by using Arnon's (1949) method.

Proline content: The proline content was estimated by the protocol given by Bates *et al.* (1973).

Estimation of protein content: The protein content was estimated by Bradford method (1976).

Leghaemoglobin content: Leghaemoglobin content was estimated by the protocol given by Bergersen method (1980).

Estimation of nitrogen content: Total nitrogen content was estimated by the method adopted by Snell and Snell (1967).

Phosphorus content: Total phosphorous was estimated by Olsen method (1954).

Determination of Nitrate reductase: Enzyme nitrate reductase activity was estimated by Hageman method (1980).

Determination of Nitrite reductase: Enzyme nitrite reductase activity was estimated by Guerrero method (1982).

RESULT AND DISCUSSION

Chlorophyll: Highest chlorophyll content (2.679) was observed in 25 gm/m^2 fly ash treated plot as compared to control (2.326) and other treatments (2.470, 2.450) respectively. Photosynthetic pigments (Chlorophyll a and b) increases with use of lower amount (25gm/m^2) of fly ash in soil (Table-1). Application of higher amount of fly ash causes photosynthetic pigments inhibition due to the presence of metal ions. Metals ions generated free radicals which degrade the chlorophyll pigments. The breakdown of photosynthetic pigment may be due to replacement of Mg^{2+} ion in chlorophyll molecules by metal ions such as Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} (Kupper *et al.,* 1998). Vazquez *et al*. (1987) also reported metals possess a believable property which change the photosynthesis processes rate by disturbing the structure of chloroplast and leading to the altering in the fatty acid composition and also inhibiting photosynthetic pigment and enzymes of Calvin cycle. Similarly, Pallavi Rai (2017) was also found such kind of result in their study.

Proline: In relevant study the minimum (0.0136) proline content was found in 25gm/m^2 fly ash treated plots as compared to untreated control (0.0202) and other 50gm (0.01373) and 100gm/m^2 (0.01454) fly ash treated plots(Table-1). Increased level of proline contents in all fly ash treated plots except 25gm/m^2 treated plots indicates that fly ash induces drought stress to plants with an enhancement of proline content. Heavy metal of fly ash such as Fe, Cu, Ni, Cr, Pb, and Cd accumulate and disrupt the other metabolic pathways of plants and enhances the drought stress conditions. Fly ash (which contains Pb and Ni) is responsible for the accumulation of proline contents in treated pea plants Singh *et al,* (2012). Similar kinds of results have been also reported earlier by Rai (2017) Kumar *et al,* (2017).

Protein: In present study the maximum protein content was found in 25 gm/m^2 (0.862) fly ash treated plot as compared to control (0.851) and other fly ash treated plots (0.817, 0.836) (Table-1). It was noticed that the use of fly ash in lower amount in soil, increases the protein contents of the seed of *Pisum sativum*. However, higher amount of fly ash reduces the protein content in plants (*Pisum sativum*). Lower amount of fly ash exhibits a positive effect on protein content, it may be due to the metal catalyst such as Fe, Cu, Ni, Cr, increases the activity of proteases or other catabolic enzymes. These enzymes activate the metabolic reactions or pathways which involves in protein synthesis in seeds. Pani *et al.* (2015) were also found that protein contents significantly increases with lower amount (25%) of fly ash application. However, reduction in the protein content was also reported under higher amount of fly ash (Dash *et al.,* 2017). Pandey *et al.,* 2010, were also reported, application of access amount of fly ash causes decrement in total protein content due to presence of metal ions or degradation of chlorophyll by free radical generated by metals.

Leghaemoglobin: The highest leghaemoglobin content was observed in 25 gm/m^2 treated plots with fly ash as compared to control and other (50 gm and 100 gm/m^2) fly ash treated plots (Table-1). This may be due to the availability of Fe, Mo, S, and Co metals which are responsible for increasing the amount of leghaemoglobin content. These metals are used by the plant as a co-factor during the biological nitrogen fixation process. In biological nitrogen fixation process, the enzymes nitrogenase and nitrogenase reductase (from *Rhizobium* stain protein which is present in nodules) metal cluster (Mo V, and Fe). This cluster needs Fe, Mo, Co as a co-factor for nitrogenase and nitrogenase reductase enzymes activity. Further, bacteroids have a very high respiratory demand, requiring abundant cytochromes and other electron donors, each with their own Fe centers. Fly ash has the potential to provide such kinds of requisite like cofactors and electron donors in the form of metal ions Ca^{2+}, Mg^{2+}, K^+, Na^+, H^+, Al^{3+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}. Kumar *et al.* (2017) also reported that the application of fly ash in a lower amount (25 gm /m^2) increases the level of Leghaemoglobin content.

Nitrogen: Maximum nitrogen content was found in 25 gm/m^2 (0.427) and 50 gm/m^2 (0.427) fly ash treated plot as compared to untreated (0.411) and 100 gm/m^2 (0.405) fly ash treated plots (Table-2). Presence of Mo in fly ash and sufficient quantity of nitrogen present in soil and in fly ash increases the nitrate reductase enzyme activity (Irfan Ahmad *et al.,* 2017). Nitrate reductase activities vice versa enhance the nitrogen content in leaves. So the nitrogen content is enhancing by the lower amount of fly ash (Kumar *et al.,* 2017). Sarangi and Mishra, 1998 also reported availability of As (arsenic) (which is present in coal fly ash) in fly ash cause the symptoms of phytotoxicity and decrease the initial growth of young black gram plants. This is the factor of a decline in nitrogen amount with enhancement of the concentration of fly ash. Irfan Ahmad in 2017 was also suggests that fly ash contained very low amount of nitrogen, which increases the activity of nitrate reductase enzyme.

Phosphorus: In present investigation the highest phosphorous content was observed in 50 gm/m^2 fly ash treated plot as compare to control and other (25 gm, 100 gm/m^2) fly ash treated plots (Table-2). Coal fly ash when applied in large amounts than site-specific requirements adverse effects are inevitable. Shaheen *et al.* (2014) were also reported that in such conditions, inhibition of plant growth could result from toxicity of metals and lower availability of nitrogen (usually present in Coal fly ash in any significant amounts) and phosphorus (coal fly ash contain considerable amounts of phosphorous but is not in a forms readily available to plants).

Nitrate reductase activity:

Nitrate reductase is capable of utilizing the reduced from of pyridine, nucleotide, flavins as an electron donor for reduction of nitrate to nitrite. NADH dependent nitrate reductase is most prevalent in plants. The activity of nitrate reductase (at 65 days) was significantly decreased in compare to control and all fly ash treated plots (Table-2). This may be due to the nitrate reductase activity indirectly affected by the presence of Phosphorous. Phosphorous is involved in phosphorylation and change in simple sugars towards respiration as a result; oxidation of photosynthates makes more reducing power subsequently for nitrate-mediated NO_3^- reduction. Presence of Mo in fly ash and sufficient quantity of nitrogen and phosphorous present in soil and in fly ash increases the nitrate reductase enzyme activity (Irfan *et al.,* 2017). Similar kinds of results have also been reported earlier by Qurratul (2014), and Melavanki (2017).

Nitrite reductase activity: In present study it was observed that the nitrite reductase activity (at 65 days) was gradually inhibited in all the treated plots as compared to untreated plot (Table-2). It may the reason, nitrate and nitrite reductase enzymatic activities enhanced in starting 10 to 20 days after sowing and inhibited thereafter (Melavanki, 2012) because of the senescence of nodulation. Presence of Mo in fly ash and sufficient quantity of nitrogen in soil and in fly ash increases the nitrate reductase enzyme activity in starting 35 days of sowing. Irfan Ahmad in 2017 was also suggests that, the fly ash contained very low amount of nitrogen, which increases the activity of nitrate reductase enzyme in starting days of sowing.

TABLES

Table 1 – Effect of fly ash amended soil on chlorophyll, proline, protein and leghemoglobin contents of pea plants.

Treatments	Chlorophyll (mg/gm)	Proline (mg/gm)	Protein (mg/gm)	Leghemoglobin (mg/g fresh weight)
Control	2.326	0.0202	0.851	0.0208
25gm/m^2 fly ash	2.679	0.0136	0.862	0.0229
50gm/m^2 fly ash	2.470	0.0137	0.817	0.0170
100gm/m^2 fly ash	2.450	0.0145	0.836	0.0175

Table 2 – Effect of fly ash amended soil on phosphorus, nitrogen, nitrate, and nitrite reductase enzymes activity of pea plants.

Treatments	Nitrogen (mg/gm)	Phosphorus (mg/gm)	Nitrate reductase (μM/min/mg Protein)	Nitrite reductase (μM/min/mg Protein)
Control	0.411	0.558	0.234	0.979
25gm/m^2 fly ash	0.427	0.568	0.174	0.648
50gm/m^2 fly ash	0.427	0.604	0.174	0.602
100gm/m^2 fly ash	0.405	0.365	0.230	0.518

CONCLUSION:

The present study was conducted to assess the stimulating impact of fly ash on biochemical parameters of *Pisum sativum* such as photosynthetic pigment, protein, leghaemoglobin, nitrogen, phosphorous and some nitrogen fixing enzymatic capacity. After analyzing all the results of experiment it was demonstrated that impact of fly ash to a optimized amount results in an increase in availability of macro- and micro nutrient of the soil. The biochemical parameters were significantly increased except proline in 25gm/m^2 treated plots. Low doses of fly ash (up to 25gm/m^2) did not cause significant increase in heavy metal concentration and could be used as soil manure or fertilizer. Fly ash acts as an excellent soil modifier conditioner 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. At the same time it will be safe and eco-friendly disposal option for huge amount of fly ash.

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