Plant Archives Vol. 19 No. 2, 2019 pp. 2959-2963 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

VARIATIONS IN MORPHOLOGICAL PARAMETERS AND PIGMENT CONTENT OF *CALENDULA OFFICINALIS* GROWN IN FLY ASH AMENDED SOIL

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Abstract

Fly ash is a major environmental contaminant produced from coal combustion in thermal power stations. Out of the various possible applications of fly ash, one of the major use is in agriculture for another green revolution. The present study explores the effect of fly ash amendments on the growth and photosynthetic pigment content of pot marigold (*Calendula officinalis*). Pot cultures were done using control, 10%, 20%, 40%, 60%, 80% and 100% fly ash amended soil ratios. Photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophyll and carotenoids and morphological parameters viz. plant height, leaf length, number of leaves per plant, leaf width and leaf area were studied. It was observed that 40% fly ash-soil ratio tends to support the maximum growth and photosynthetic pigment content in *Calendula officinalis*. Soil amended with 10%, 20%, 40% and 60% of fly ash exhibited a better plant growth performance and an enhanced pigment content as compared to 80% and 100% fly ash in soil with respect to control.

Key words : Fly ash, Soil, Plant growth, Photosynthetic Pigment, *Calendula officinalis.*

Introduction

Fly ash is an inorganic waste produced as a fine particle deposit by the coal combustion process. It is the major contaminant produced from different thermal power plants. As a fine particle residue, its size ranges from 0.01 to 100 mm and due to thin weight it is carried out via the flue gases from boiler chimneys (Kumari and Prasad, 2014). Fly ash is of two types: Class C and Class F depending upon the kind of coal being used for combustion process. In India, approximately 100 million tonnes of fly ash is produced annually from 85 thermal power plants and is projected to reach more than 175 million tonnes in the coming future (Katiyar *et al.,* 2012). However, it has already reached to 170 million tonnes in the year 2017 while its utilization has not even reached to 50% of its production. Therefore, there is a need to employ methods and technologies in order to utilize fly ash in various sectors including industries, infrastructure, and agriculture.

Fly ash comprises of micro and macro-elements which are beneficial for the plant growth including some toxic metals. It is alkaline in nature and its use in agricultural fields as an amendment in soil, proves to be a better soil nourishment. (Kishor *et al.,* 2010). Fly ash contains almost all nutrients beneficial for the plant growth but is deficient in nitrogen and phosphorus due to this reason its high application in agriculture can produce deleterious effect to soil as well as plant (Dahiya and Budania, 2018). The toxic heavy metals present in the soil due to fly ash amendment have a negative impact on microbial population and soil fertility and thereby affect the plant metabolism (Singh *et al.,* 2010). Fly ash utilization in agricultural fields can improve the soil texture, water holding capacity of soil, organic matter content of soil and neutralize the soil pH and bulk density (Raj and Mohan, 2014; Raj *et al.,* 2015). Addition of fly ash to soil changes the physical, chemical, and biological properties of soil and thereby enhances the plant growth (Sheoran *et al.,* 2014).

Use of fly ash in agricultural fields as a soil amendment has been observed by various scientist for improving the soil texture and plant growth which includes

Helianthus annuus (Pani *et al.,* 2015), *Capsicum annuum* (Thakare *et al.,* 2013), *Oryza sativum* and *Zea mays* (Panda *et al.,* 2015) and green gram (Nashine, 2014). In regards to different studies conducted on plant growth improvement with respect to fly ash amended soil, it seems that although fly ash has various applications in agriculture as a base material, but due to the heavy metal and toxic elements at high application rate of fly ash, keen use of fly ash in agriculture is highly important to understand.

Till now, no significant data has been reported on the utilization of fly ash in cultivation of *Calendula officinalis* plant and determining its impact on plant growth performance. The present work includes utilization of fly ash in different combination of soil- fly ash doses for cultivating *Calendula officinalis* to find out the best possible dose for its high yield and productivity. It also includes studying the variations in morphological parameters and photosynthetic pigment content of the cultivated plant in different doses of fly ash soil amendments.

Material and Methods

Collection of Fly ash and soil

The fly ash used in the study was collected from the NTPC, Dadri, Uttar Pradesh, India and garden soil (without any added manure) was collected from the Nursery, Sector-18, Noida, Uttar Pradesh. The fly ash and soil sample were air dried and sieved before use.

Experimental Set-up

Pot experiment was conducted during the winter season of 2018 at Organic farm of Amity University, Noida. Earthen pots having the diameter of 25 cm were used for the experiment and the experimental design was carried out from November to April end. Different amendments of fly ash and soil were prepared by mixing them in different w/w ratios 10%, 20%, 40%, 60%, 80%, 100% fly ash in soil and 100% soil was taken as control to compare the results. The above experimental design were coded as- T_1 (Control), T_2 (10%), T_3 (20%), T_4 (40%), T_5 (60%), T_6 (80%) and T_7 (100%). For each treatment, the sample size was 20 and the total amendment set of pots were seven.

Calendula seeds were sown in pots at a depth of 0.5 cm and irrigated carefully. The plants were irrigated with tap water with an interval of 2 days. The plants were grown for a period of six months (as per their life cycle). After 90 days of growth, plant leaves from each pot were harvested to record and study the morphological and biochemical parameters.

Determination of Morphological parameters

The plant leaves were harvested and washed carefully to remove the dust particles from the surface and further blotted softly to dry on a blotting paper. Plant height, leaf length, number of leaves per plant, leaf width and leaf area from different treatment pots were measured and recorded at 90 days of growing season. A total of 15 replicates were studied for each concentration and mean value was taken for calculation.

Estimation of pigment content

The photosynthetic pigment content such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content of fresh leaf samples were measured at four different absorbance- 480 nm, 510 nm, 645 nm and 663 nm following the methods of Hiscox and Israelstam, 1976.

Calculations were made according to the following equation-

$$\text{Chl a (mg/g fresh weight)} = \frac{12.3\times\text{O.D. at 663 nm} - 0.86\times\text{O.D. at 645 nm}}{D \times 1000 \times w} \times V$$

$$\text{Chl b (mg/g fresh weight)} = \frac{19.3\times\text{O.D. at 645 nm} - 3.6\times\text{O.D. at 663 nm}}{D \times 1000 \times w} \times V$$

$$\text{Carotenoids (mg/g fresh weight)} = \frac{7.6\times\text{O.D. at 480 nm} - 1.49\times\text{O.D. at 510 nm}}{D \times 1000 \times w} \times V$$

$$\text{Total Chlorophyll (mg/g fresh weight)} = \frac{20.2\times\text{O.D. at 645 nm} - 8.02\times\text{O.D. at 663 nm}}{D \times 1000 \times w} \times V$$

Where,

V= Volume of Extract, w= Weight of the leaf material, D= length of the path length

Results and Discussion

Morphological parameters

The study on effect of fly ash amended soil on different morphological parameters of *Calendula officinalis* revealed that the observation on plant height, number of leaves, leaf area, leaf width and leaf height significantly differ due to treatment combinations of both fly ash and soil. Plant growth and development analysis is a necessary step towards understanding plant performance and productivity under different conditions (Parween *et al.,* 2011a). The observations clearly indicate the positive influence of pot mixtures at low fly ash doses in enhancing different growth parameters however the results are suppressed as the concentration of fly ash in soil is high (Fig. 1). In the winter season (December 2018 to April 2019), the plant height (12.24 cm), number of leaves (11.4), leaf area (25.8 cm^2), leaf width (3.42 cm) and leaf length (11.98 cm) in treatment T4 were recorded maximum at 40% fly ash-soil concentration and plant height (8.63 cm), number of leaves (7.2), leaf area (12.3 cm^2), leaf width (2.14 cm) and leaf length (8.42 cm) were recorded minimum in treatment T7 *i.e.* 100% fly ash concentration with respect to control plant height (10.7 cm), number of leaves (9.3), leaf area (17.9 cm^2), leaf width (2.77 cm) and 10.22com of leaf length (Table 1).

The present study demonstrated that lower concentrations of fly ash seem to be effective, non-phytotoxic and hence increase the plant growth performance and productivity up to a limit however the higher amendment levels appear to be toxic due to which plant height, number of leaves, leaf area, leaf width and leaf height are reduced. Our results are in accordance with other studies which demonstrate enhanced plant growth under low application rates (Katiyar *et al.,* 2012; Singh *et al.,* 2012). As Fly ash can induce changes in the chemical properties of the soil due to the availability of toxic metals in higher concentration, hence this might be the cause of deleterious effect at higher application rates (Maiti and Prasad, 2016). Experiments conducted both under field and green house conditions with fly ash amendments of soil showed that different chemical constituents of fly ash can promote plant growth and improve the agronomic properties of the soils at lower concentrations (Singh *et al.,* 2012). Similarly Pandey et al. (2009) observed that addition of limited concentration of fly ash to soil shows positive results in various parameters of plant growth and yield. Similar findings have been reported by various scientists on different crops and vegetables like *Zea mays*, *Helianthus annus*, *Solanum tuberosum, Brassica oleracea*, *Sorghum bicolor, Echinochola crusgalli, Daucus carota, Allium cepa, Phaseolus vulgaris, Triticum aestivum (*Aggarwal *et al.,* 2009; Siddiqui and Singh, 2005). Presence of essential major and micro nutrients in fly ash makes it an important source of plant nutrients essential for plant growth and development though higher concentration can be toxic in the food chain.

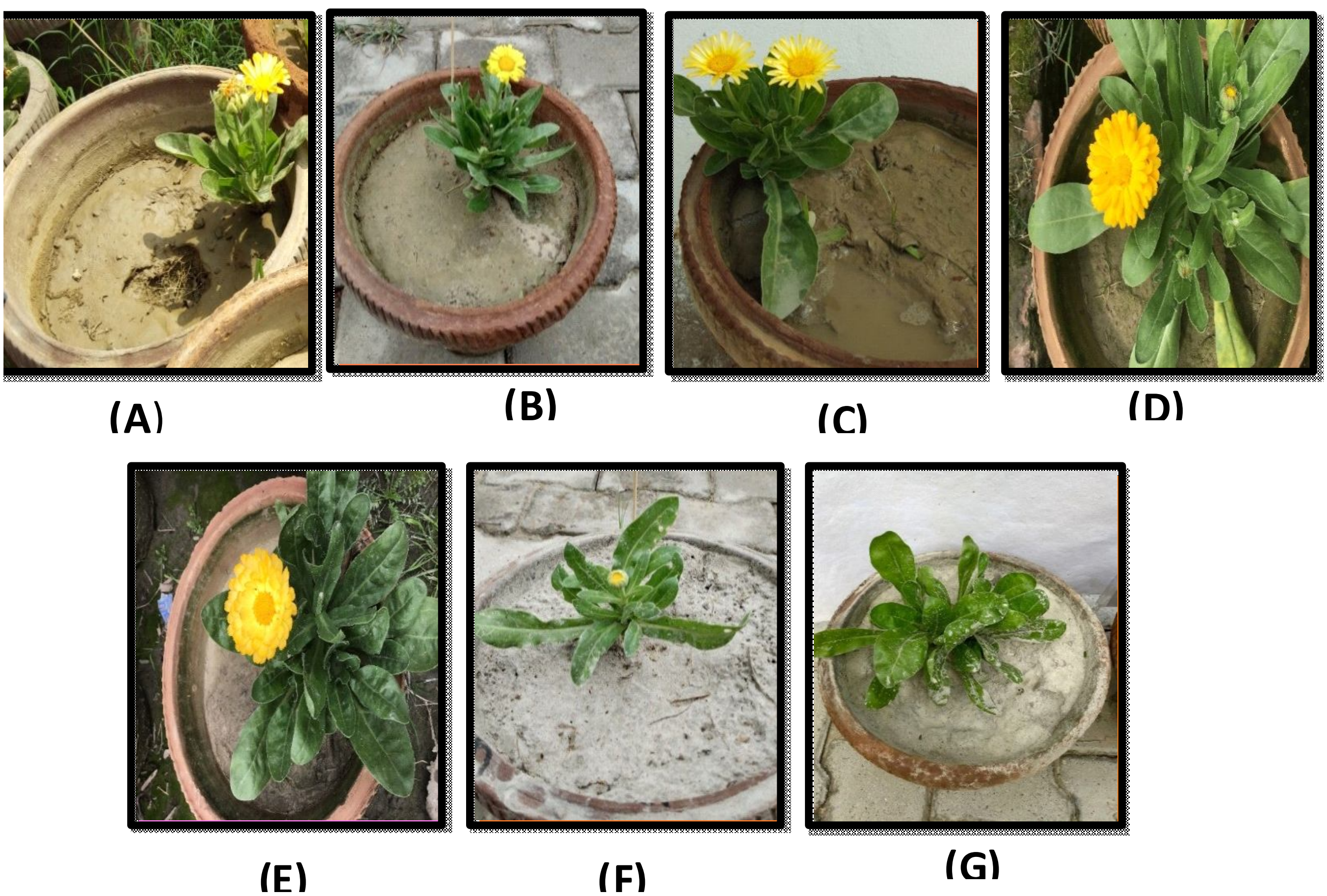

Fig. 1: Growth performance of *Calendula officinalis* in fly ash amended soil. (A) : Control (only soil), (B) : 10% Treatment (10% fly ash and 90% soil), (C) : 20% Treatment (20% fly ash and 80% soil), (D) : 40% Treatment (40% fly ash and 60% soil), (E) : 60% Treatment (60% fly ash and 40% soil), (F) : 80% Treatment (80% fly ash and 20% soil) and (G): 100% Treatment (fly ash only).

Pigment content

Generally, photosynthetic pigment content depends upon the leaf area, stomatal response and the prevailing environmental conditions (Kausar *et al.,* 2015). Plant tolerance and sensitivity to heavy metals in fly ash is reflected by the level of pigment (chlorophyll and carotenoid) content. In the present study, chlorophyll a, b, carotenoids and total chlorophyll content recorded to be increased under low concentration of fly ash, but decreased under high concentration of fly ash. The highest pigment content in the leaves of *Calendula officinalis* was observed in 40% fly ash concentration as shown in table 2. Chlorophyll a (1.36 mg/g), chlorophyll b (0.54 mg/g), total chlorophyll (1.78 mg/g) and carotenoids (0.58 mg/g) in treatment T4 were recorded maximum at 40% fly ash-soil treatments and chlorophyll a (0.92 mg/g), chlorophyll b (0.28 mg/g), total chlorophyll (1.25 mg/g) and carotenoids (0.29 mg/g) in treatment T7 were recorded minimum at 100% fly ash-soil treatments with respect to control T1 chlorophyll a (1.03 mg/g), chlorophyll b (0.36 mg/g), total chlorophyll (1.38 mg/g) and carotenoids (0.34 mg/g).

The pigment parameters significantly increased in the treatments T2, T3, T4 and T5 as compared to the control (T1), although decreased at higher concentrations of T6 and T7. Chlorophyll is synthesized by plant effects its overall growth (Sharma *et al.,* 2010). The increase of Chlorophyll content may be due to limited concentrations of heavy metals in fly ash. According to Liang *et al.,* 2012, the low dose of Cd and Pb enhanced photosynthetic activity of *Jatropha curcas*. The excess of heavy metals (Cd, Pb, Cr and Cu) inhibits the plant growth of *Jatropha curcas*. In this research the slight stress of heavy metals decreased all biochemical contents (Devi Chinmayee *et al.,* 2014). This is due to the inhibition of carotenoid and chlorophyll biosynthesis and reduction in the co-ordination of these pigments with photosystems. The results at high doses might have occurred to protect the plant against the toxic effect of free radicals which is supported by the studies on pigment content of *Solanum nigrum* L. and *Solanum melongena* grown on different fly ash doses (Robab *et al.,* 2010; Gond *et al.,* 2013) Accumulation of heavy metals at high concentration of fly ash leads to the inhibition of chlorophyll content (Sharma *et al.,* 2010)

Conclusion

Coal based thermal power plants cause environmental pollution and one of the main issue of concern is fly ash disposal from thermal power plants. Therefore, in an approach for the proper waste management, fly ash can be used as a soil ameliorant and as a nutrient supplement thereby solving solid waste problem to some extent. An ultimate goal is to utilize fly ash in agriculture to a certain extent to achieve enhanced fertility and plant yield without affecting the soil quality and reducing the accumulation of toxic heavy metals in plants. The present study demonstrates stimulating effects of fly ash on growth and photosynthetic pigments in *Calendula officinalis* growing on soil amended with low concentration levels of fly ash. Higher concentration of fly ash however

Table 1: Effect of fly ash on different morphological parameters of *Calendula officinalis* in different soil-fly ash treatments.

Parameters	T_1 (control)	T_2 (10%)	T_3 (20%)	T_4 (40%)	T_5 (60%)	T_6 (80%)	T_7 (100%)
Plant Height (in cm)	10.7±1.49	10.91±1.77	11.26±0.86	12.24±0.88	12.06±2.19	9.07±1.62	8.63±1.62
No. of Leaves/plant	9.3±1.9	10.2±2.36	10.4±2.76	11.4±1.69	10.5±2.91	8.1±2.12	7.2±2.48
Leaf area (in cm²)	17.9±3.14	22.3±3.16	24.6±4.43	25.8±3.75	25.4±5.33	15.2±3.76	12.3±2.41
Leaf Width (in cm)	2.77±0.29	3.18±0.36	3.28±0.32	3.42±0.29	3.41±0.43	2.16±0.37	2.14±0.37
Leaf Length (in cm)	10.22±1.69	10.74±2.29	10.86±1.01	11.98±1.37	11.24±1.93	9.89±1.28	8.42±1.61

T=Treatment, T_1 (Control): 100% soil, T_2 (10%): 10% fly ash+ 90% soil, T_3 (20%): 20% fly ash+ 80% soil, T_4 (40%): 40% fly ash+ 60% soil, T_5 (60%): 60% fly ash+ 40% soil, T_6 (80%): 80% fly ash+ 20% soil and T_7 (100%):100% fly ash. All values represent mean ± SD where the sample size (n= 15).

Table 2: Effect of various soil- fly ash doses on photosynthetic pigments- chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content (mg/g fresh weight) in leaves of *Calendula officinalis.*

Parameters	T_1 (control)	T_2 (10%)	T_3 (20%)	T_4 (40%)	T_5 (60%)	T_6 (80%)	T_7 (100%)
Chlorophyll a	1.03±0.016	1.17±0.139	1.25±0.026	1.36±0.142	1.28±0.031	1.01±0.003	0.92±0.002
Chlorophyll b	0.36±0.034	0.45±0.042	0.49±0.012	0.54±0.024	0.51±0.047	0.34±0.026	0.28±0.021
Total Chlorophyll	1.38±0.051	1.42±0.072	1.68±0.054	1.78±0.074	1.70±0.068	1.36±0.043	1.25±0.021
Carotenoids	0.34±0.029	0.39±0.043	0.47±0.056	0.58±0.041	0.51±0.054	0.32±0.023	0.29±0.037

T=Treatment, T_1 (Control): 100% soil, T_2 (10%): 10% fly ash+ 90% soil, T_3 (20%): 20% fly ash+ 80% soil, T_4 (40%): 40% fly ash+ 60% soil, T_5(60%): 60% fly ash+ 40% soil, T_6 (80%): 80% fly ash+ 20% soil and T_7 (100%):100% fly ash. All values represent mean ± SD where the sample size (n= 15).

reduced plant performance and showed detrimental effects of fly ash.

Acknowledgement

Authors greatly acknowledge the financial support provided by UPCST funding agency (sanction letter no. CST/AAS/D-4995). Authors also would like to thank Amity University, Noida for providing the laboratory facilities to carry out this work.

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