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Influence of Fly Ash and Growth Regulator with Soil for Determination of Chlorophyll in *Arachis hypogaea* L.

Shweta Sao[1], Pankaj K. Sahu[2]

[1]Department of Microbiology, Dr. C. V. Raman University Kota, Bilaspur, India; [2]Department of Botany, Dr. C. V. Raman University Kota, Bilaspur, India.
Email: sahu.pankaj1@gmail.com

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ABSTRACT

The present investigation was conducted to find out the effect of varying levels of fly ash and growth hormones on the determination of chlorophylls. The experiments were conducted in pots during 2009-2010 with *Arachis hypogaea* L. (groundnut) grown with different levels of fly ash concentration, and soil was used (various combinations) at Guru ghasidas University, Bilaspur (CG.) India. In fresh leaf, chlorophylls content varies in the plain soil from 0.29 to 0.64 mg g^{-1}, which is less for photosynthetic activities. *Arachis hypogaea* L. showed maximum germination percentage, increasing leaf area, enhancement of root & shoot length, whereas Fly ash, bio fertilizers with growth hormone showed minimum values in all parameters. Results showed that, for combination of A to E, the value of chlorophyll ranged from 0.270 mg g^{-1} to 0.395 mg g^{-1}, and chlorophyll b ranged from 0.400 mg g^{-1} to 0.489 mg g^{-1}, whereas fro total chlorophyll ranged from 0.67 to 0.85 mg g^{-1}. In the present work, chlorophyll a, chlorophyll b & total chlorophyll content in fresh leaf, after 45 days, were recorded as 0.395 mg g^{-1}, 0.489 mg g^{-1} and 0.851 mg g^{-1} while in 90 days were recorded as 10.38 mg g^{-1}, 0.48 mg g^{-1} and 0.86 mg g^{-1} respectively, in less amount combination of fly ash, soil content with application of growth hormone.

Keywords: *Arachis hypogaea*; Growth Hormone; Soil; Chlorophyll; Fly Ash

1. Introduction

Arachis hypogaea L. (Groundnut) is a small branched herb, erects or trails on the ground and bears small yellow flowers. The cylindrical reticulated two seeds pod within the outer shell. Increased concentration of cement dust pollutants causes invisible injuries like progressive decline in the physiological process such as photosynthetic ability and respiration rate of leaves. *Arachis hypogaea* L. can adapt to a variety of soil and climatic conditions and the use of fly ash as a liming agent in mono and dicotyledonous plants for better crop yields. Fly ash has tremendous potential as a nutrient supplement and plays a favourable role in increasing growth and yield of ground nut [1-5]. Fly ash has similar physicochemical properties with soil. It can mix homogeneously and can improve agronomic properties of soil [6]. Fly ash is the treasure of trace elements. It makes the trace element readily available to the crop when mixed with soil [7,8]. Auxin increased respiration rates are suggestive of parallel relationship of growth, respiratory activity and found to increase RNA synthesis in tissue of higher plants [9]. Use of fly ash ameliorates soil acidity for maximum uptake of trace elements from fly ash which acted as a reserve of trace element when mixed in soil. Fly ash helps to retain water in the soil and also helped CO_2 evolution. The plant hormone Indole acetic acid and Gibberellic acid helped protein, oil synthesis and also increased respiration rate. Soil metabolic activities, activities of amylase invertase and protease, chlorophyll a & b, carotenoid and protein content are increased in fly ash amended soil [10-14].

Cement dust pollutants blocked the stomata, reduction in number of annual crops and due to cement dust decreased the productivity and concentration of chlorophyll in a number of crops reported [15,16]. The reduction in protein, starch, yield and phytomass of groundnut occurred due to cement dust *Arachis hypogaea* L. [17]. Protein synthesis decreased due to the low chlorophyll and reduced leaf area surface similar findings were reported [18]. Remunerative responses of groundnut crop to fer-

tilizer application have been observed both under irrigated and rain fed conditions in India [19]. Increase in groundnut yield due to the application of NPK was also reported [20].

2. Material and Methods

Determination of chlorophyll a, b and total chlorophyll [21], Chlorophyll is extracted in 90% acetone from 1 gm leaves of *Arachis* (systronics-20). Using the absorption coefficient, the amount of chlorophyll a, b and total chlorophyll were estimated in the leaves of *Arachis hypogaea* L. 1 gm of well mixed representative sample of leaves were finely cut and ground with 20 ml of 80% acetone, centrifuged (5000 rpm 5 minutes) and supernatant liquid was transferred to a 100 ml volumetric flask. The procedure was repeated till residue become colourless. Volume was made up to 100 ml mark with 80% acetone in all the three cases individually.

The amount of Chlorophyll was calculated using the formula mentioned below:

$$\text{Mg chlorophyll a/gram of leave} = 12.7\ (\text{A615}) \times \text{V/1000} \times \text{W}$$

$$\text{Mg chlorophyll a/gram of leave} = 12.7\ (\text{A615}) \times \text{V/1000} \times \text{W}$$

$$\text{Mg total chlorophyll /gram of leaves} = 20.2(\text{A645}) - 8.02(\text{A} \times 663) \times \text{V/1000} \times \text{W}$$

where, A = Absorbance at specific wave lengths, V = Final volume of chlorophyll extract in 80% acetone, W = Fresh weight of leaves extracted.

Chlorophyll a and b, Carotenoids were extracted from the leaves and estimated by the method of Arnon (1949). Absorbance was measured at 645, 663 and 480 nm with a spectrophotometer (U200 1Hitachi) against 80 per cent acetone as blank. Chlorophyll content was calculated using the formula proposed by Arnon (**Table 1**).

3. Result and Discussion

In the present work chlorophyll a, chlorophyll b and total chlorophyll in fresh leaf were observed as 0.35, 0.29 and 0.64 mg g^{-1} respectively while after 45 days chlorophylls were estimated as 0.395 mg g^{-1}, 0.489 mg g^{-1} and 0.851 mg g^{-1}. The value of chlorophyll a was observed as 0.270 mg g^{-1}, 0.280 mg g^{-1}, 0.300 mg g^{-1}, 0.377 mg g^{-1} and 0.395 mg g^{-1} in the set A, B, C, D and E respectively, whereas for chlorophyll b the value was recorded as 0.400 mg g^{-1}, 0.454 mg g^{-1}, 0.460 mg g^{-1}, 0.480 mg g^{-1} and 0.489 mg g^{-1} respectively. For total chlorophyll it was 0.670 mg g^{-1}, 0.734 mg g^{-1}, 0.760 mg g^{-1}, 0.848 mg g^{-1} and 0.851 mg g^{-1} respectively (**Figure 1**).

In plain soil, chlorophyll a, chlorophyll b and total chlorophyll were present as 0.35, 0.29 and 0.64 mg g^{-1} in fresh leaf, respectively, which is less for photosynthetic activities. In the present work chlorophylls were present in 10.38 mg g^{-1}, 0.48 mg g^{-1} and 0.86 mg g^{-1} fresh leaf after 90 days. For A to E treatment chlorophyll values were 0.270 mg g^{-1}, 0.280 mg g^{-1}, 0.300 mg g^{-1} and 0.377 mg g^{-1} fresh leaf, respectively. For chlorophyll b, values were 0.400 mg g^{-1} fresh leaf, 0.454 mg g^{-1} fresh leaf, 0.460 mg g^{-1} fresh leaf and 0.480 mg g^{-1} fresh leaf, respectively and total chlorophyll in combination A was found to be 0.67 mg g^{-1} fresh leaf and B—0.73 mg/gm fresh leaf and C—0.76 mg g^{-1} fresh leaf, and in combination D, 0.85 mg g^{-1} fresh leaf (fig). Treatments with ABA and GA_3 significantly increased the total chlorophyll contents in *Mentha* plants. Similar results were observed that in Paclo butrazol treated barley and carrot and Paclobutrazol treated leaves were dark green due to high chlorophyll content in potato [22-24]. In combination of 30% FA + 70% Soil + GH, *Arachis hypogaea* L. showed maximum germination %, Increasing leaf area, and enhancement of shoot length, whereas Fly ash + NPK + GH showed minimum all parameters.

Gibberellic acid increased the vegetative growth and pigment concentration in maize [25]. Foliar application of GA_3 improved the chlorophyll levels in salinity stressed maize plants [26]. These results suggested that plain soil could not contribute to chlorophyll formation. It was performed that the addition of NPK had increased chlorophyll formation by producing nitrogen and nutrient management for sustainable groundnut productivity in India [27-30]. Mg has contributed its best role in structural constitution of chlorophyll a and b, and its absence causes early derangement of chloroplast structure [31], while Fe is contributing its important role to chlorophyll synthesis [32]. Nutrient management for the sustainable productivity in India was performed that fly ash and growth hormone increased the oil content in linseed crop [33,34]. IAA and GA hormones increase the germination percentage [35] while the use of IAA and GA hormone regulates the biochemical process of the plant, which results higher percentage of oleic and oleic acid content [36]. IAA and GA increase the stem and root elongation and the plant growth parameters like root length, shoot length, number of leaves, leaf area increase, and seed contents also increase. Increasing number of leaves and leaf area increases the level of the chlorophyll a & b [37]. Growth biochemical and yield responses of groundnut are due to the effect of cement dust deposition [17].

3.1. Effect of Plant Growth Regulators on Chlorophyll Content

The total chlorophyll contents of the leaves increase with the age in control and treated Mentha leaves. The maximum increase was found on 90 DAP in DIZ treatments and it was 132.91 percent over control. Among the treat-

Table 1. Effect of various combinations with fly ash in leaves formation, root & shoot growth.

S.N.	Combinations	Pot number	% Germination	Number of Leaves	Leaf area (cm^2)	Shoot length (cm)
				In 45 days	In 45 days	After 45 days
1.	Fly ash 100%	1,2,3	-------	------	------	-------
2.	Plain soil 100%	4,5,6	42	243	2604.71	48.03
3.	10%FA + 90%Soil	7,8,9	42	251	2511.16	48.12
4.	20%FA + 80%Soil	10,11,12	41	229	2641.70	49.01
5.	30%FA + 70%Soil	13,14,15	56	235	2711.12	48.17
6.	40%FA + 60%Soil	16,17,18	54	221	2511.18	45.13
7.	50%FA + 50%Soil	19,20,21	42	220	2206.31	45.11
8.	60%FA + 40%Soil	22,23,24	33	207	2014.00	44.41
9.	70%FA + 30%Soil	25,26,27	35	211	1842.42	42.31
10.	80%FA + 20%Soil	28,29,30	30	185	1721.21	40.21
11.	90%FA + 10%Soil	31,32,33	20	164	1336.11	35.62
12.	Plan Soil + NPK	34,35,36	48	264	3001.89	50.53
13.	10%FA + 90%Soil + NPK	37,38,39	66	276	3520.95	52.46
14	20%FA + 80%Soil + NPK	40,41,42	78	306	3873.87	54.63
15.	30%FA + 70%Soil + NPK	43,44,45	60	265	3216.08	50.00
16.	40%FA + 60%Soil + NPK	46,47,48	68	246	3018.00	48.17
17.	50%FA + 50%Soil + NPK	49,50.51	42	241	2752.11	44.22
18.	60%FA + 40% Soil + NPK	52,53,54	35	217	2321.19	45.02
19.	70%FA + 30%Soil + NPK	55,56,57	24	171	2003.93	42.51
20.	80%FA + 20% Soil + NPK	58,59,60	20	125	1162.75	42.13
21.	90%FA + 10%Soil + NPK	61,62,63	10	101	1151.52	50.22
22.	10%FA + 90%Soil + NPK	64,65,66	45	234	3037.76	52.63
23.	20%FA + 80%Soil + NPK	67,68,69	78	292	3881.87	53.71
24.	30%FA + 70%Soil + NPK	70,71,72	99	327	4478.3	57.00
25.	40%FA + 60%Soil + NPK	73,74,75	78	271	3721.88	51.21
26.	50%FA + 50%Soil + NPK	76,77,78	66	265	3521.33	48.27
27.	60%FA + 40% Soil + NPK	79,80,81	58	243	3321.07	45.22
28.	70%FA + 30% Soil + NPK	82,83,84	40	221	3516.24	43.88
29.	80%FA + 20% Soil + NPK	85,86,87	41	207	3361.23	43.28
30.	90%FA + 10%Soil + NPK	88,89,90	20	142	2321.21	38.33
31.	10%FA + 90% Soil + GH	91,92,93	50	237	2711.22	50.92
32.	20%FA + 80% Soil + GH	94,95,96	54	262	2822.61	52.92
33.	30%FA + 70% Soil + GH	97,98,99	60	254	3416.88	54.13
34.	40%FA + 60% Soil + GH	100-102	58	242	3092.12	52.92
35.	50%FA + 50% Soil + GH	103-105	52	251	2761.73	51.52
36.	60%FA + 40% Soil + GH	106-108	41	223	2516.22	48.12
37.	70%FA + 30% Soil + GH	109-111	36	185	2014.83	45.31
38.	80%FA + 20% Soil + GH	112-114	32	161	1989.36	45.77
39.	90%FA + 10% Soil + GH	115-117	20	119	1681.14	42.83
40.	Fly ash + NPK + GH	118-120	20	72	998.31	35.45

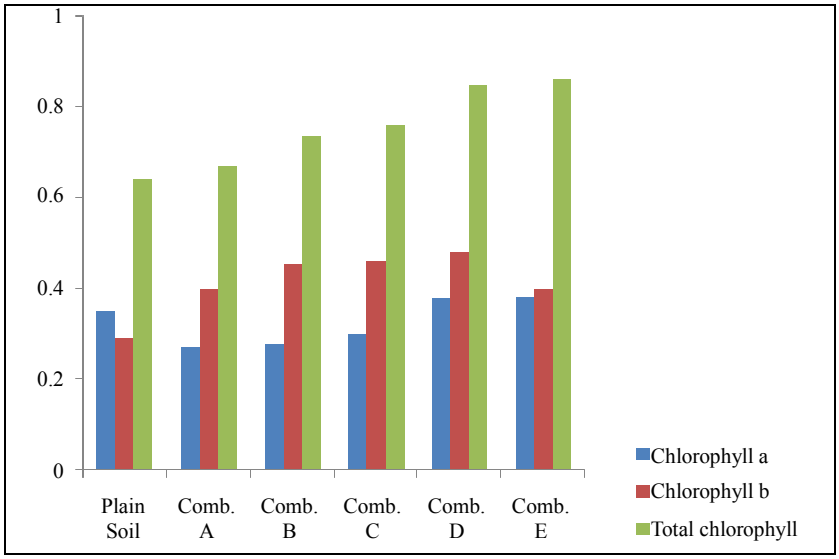

Figure 1. Showing effect of various concentrations for chlorophylls determination.

ments ABA and GA3 slightly increased and they were 123.00 and 122.11 respectively on 90 DAP.

3.2. Effect of Plant Growth Regulators on Plant Height

The total height of the plant increased with the age in the control, ABA and GA_3 treated Mentha plants, but it decreased under DIZ treatments. The increase was higher in gibberellic acid treated compared to ABA. Highest plant height was noted in 90 DAP under GA3 treatments and it was nearly 136.66 percent over control. The gibberellic acid treated plants increased the plant height. Triazole treatments reduced stem elongation and plant height in *Plectranthus forskholii*, *Manihot esculenta* and *Catharanthus roseus* [38-40]. GA_3 exerts profound effects on fundamental process of plant growth and development. GA_3 is widely regarded as a growth promoting compound that positively regulates processes such as seed germination, stem elongation and leaf expansion [41]. Split application of potassium equally at sowing and at 35 DAS increased the pod yield of groundnut, and higher availability of plant nutrients consequently had higher growth parameters in the fertilized treatments and higher yield of groundnut [42,43]. The application of 25:50:00, NPK kg ha^{-1} giving the highest plant height and total dry matter per plant at harvest and yield attributes of summer groundnut was reported [44]. Significant increase in pod yield of groundnut was observed at a fertilizer level of 30:60:30 kg NPK ha^{-1}, and the increase in yield was 30 percent higher than lower level of fertilizer doses [45]. It was observed that supplemental nitrogen either through soil or foliage at 50 and 70 DAS in addition to recommended dose of NPK increased the yield of groundnut [46]. The effect of trace elements on yield and nutrient uptake was studied in groundnut [47]. The influence of cement dust on growth and yield component of *Arachis hypogaea* popularly grew in and around the cement factory area in India.

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