

## Agronomic Biofortification of *Amaranthus dubius* with Macro Nutrients and Vitamin A

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**Abstract:** Agronomic biofortification of *Amaranthus dubius* with carbohydrates, proteins and Vitamin A using *Spirulina platensis* is reported in the present preliminary study. *S. platensis* was applied basally to field and its influence on germination, concentration of proteins, carbohydrates, chlorophyll, carotenoids and antioxidant activity of leaves were assessed in CO1 variety of *A. dubius*. Biofortification of the nutrients were evaluated on 5th and 20th day. Germination was optimum at 0.005 % of *S. platensis* as a fertilizer and the agronomic fortifying agent. Germination in control was 82 % and 95 % in 0.005 % of *S. platensis* fortification. The concentration of total chlorophyll in control and biofortified leaf on 20th day were 85.6 mg/g and 325 mg/g of dried leaf. The concentration of proteins was 71.93 mg/g and 450 mg/g on 20th day in control and fortified leaves. *S. platensis* applied field produced leaves fortified with 1.27 % of vitamin A aldehyde. Antioxidant activity of control leaf was 34.85 % and it was significantly increased in fortified leaves to 87 %. Thus the study confirms the fortification of leaf with proteins, carbohydrates and vitamin A.

Keywords: *Amaranthus dubius*, *Spirulina platensis*, Biofortification, Vitamin A.

### 1. INTRODUCTION

Malnutrition affects millions of people every year globally. Macro and micronutrient malnutrition affect the quality of living and leads to death ultimately. Due to urbanization and industrialization, major proportion of agricultural lands is lost. The fertility of the cultivable land is progressively decreasing due to the improper management of agrochemicals. Hence the yield obtained is deficient in many macro and micro nutrients. In developing countries, more than 40% of preschool children are anemic and 38% of pregnant women are affected by micronutrient malnutrition or “hidden hunger”[1]-[2]. Protein is the macronutrient that supplies essential amino acids. WHO/FAO/UNU consultation has revised the daily average protein requirement for adult as 0.83 g/kg/d. This is 10% more than the recommendations by 1985[3]. Protein intake in rural population is insufficient in terms of digestible quality [4]. In addition to protein deficiency many preschool children suffer from Vitamin A deficiency. Globally about one third of pre-school



children are suffering from vitamin A deficiency [5]. Biofortification for macro and micronutrients is an attractive tool to improve the nutritional status of food. It can be accomplished by traditional plant breeding, recombinant technology and agronomic practices. Success of biofortification depends on the type of crop being fortified, its yield attributes and consumption by vast majority of population, simplicity of the measures to be adopted for fortification and economic benefit assured to the farmers. *S. platensis* is rich in proteins, carbohydrates, lipids, minerals, vitamins, essential fatty acids, phenolic acids and tocopherol. From Mayan Civilization onwards *S. platensis* is consumed as a food. In addition to its nutritional significance, *S. platensis* is well known for its antioxidant, anticancer and immuno modulatory

activities [6]. In spite of its nutritional and medicinal significance, the consumption is very low because of its unpleasant unique odor. Biofortification of a leafy vegetable with the nutrients from

*S. platensis* is an attractive solution, as the quantity of leaf consumed is more and bioaccumulation in

leaf is greater than other parts of the plant. *Amaranthus dubius* belongs to the economically important family *Amaranthaceae*. The leaves of the plant are recommended as a good food with medicinal properties for young children, and lactating mothers. It grows under any type of soil. It has the high photosynthetic rate [7]. Hence, the objective of the present study is to fortify *A. dubius* with protein, carbohydrates, carotenoids, vitamin A and antioxidants by exploiting *S. platensis* as a fertilizer and a fortifying agent.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All chemicals were purchased from High media, Mumbai, India. Solvents used were of analytical grade. The seeds of *A. dubius* (CO 1) was purchased from the local market.

### 2.2 Preparation of field for plant growth

The black soil in the College campus was used for the field trial. Two weeks before the sowing of seeds, the field was ploughed well and used. Two plots of 4x4 feet were prepared in completely randomized block design. All treatments were executed in triplicates. One plot served as a control where no fortification was done. The treatment plot included fortification with 5g of *S. platensis* flakes as a basal application. Leaf samples were collected on 5th and 20th day for the analysis of total carbohydrates, proteins, chlorophyll, carotenoids and antioxidant activity. The leaf samples on 20th day were analyzed by GC-MS.

### 2.3 Biochemical analysis of leaves

Seed germination was determined by the method of ISTA [8]. Total carbohydrates was estimated by the method of Hodge and Hofreiter [9]. total chlorophyll and carotenoids were estimated by the method of Arnon [10] and proteins by Lowrys method [11]. Total antioxidant activity of the leaves were assessed by using DPPH assay [12].

### 2.4 GC-MS of *A. dubius* leaves

The ethanol extract of leaves were used for GC-MS analysis in Clarus 500 Perkin Elmer. Elite-5MS column was used. The flow rate of carrier gas was maintained at 1ml /min.

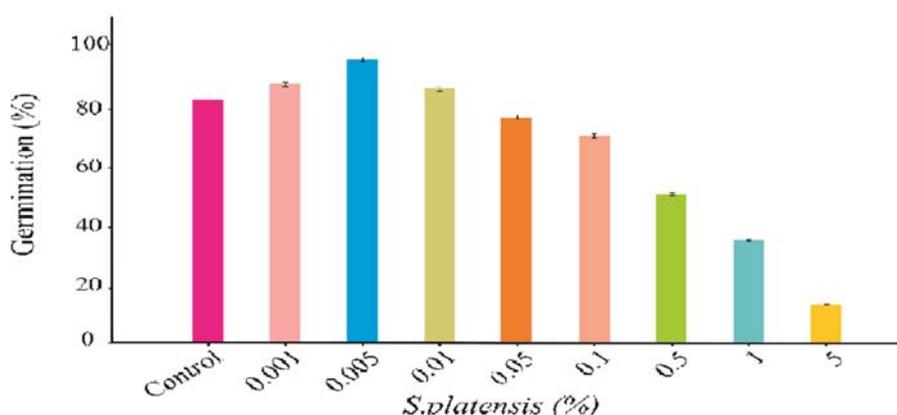
### 2.5 Statistical analysis

All the experimental were replicated thrice. The results are expressed is the mean  $\pm$  standard error.

## 3.RESULTS AND DISCUSSION

### 3.1. Influence of *S. platensis* on Germination of Seeds

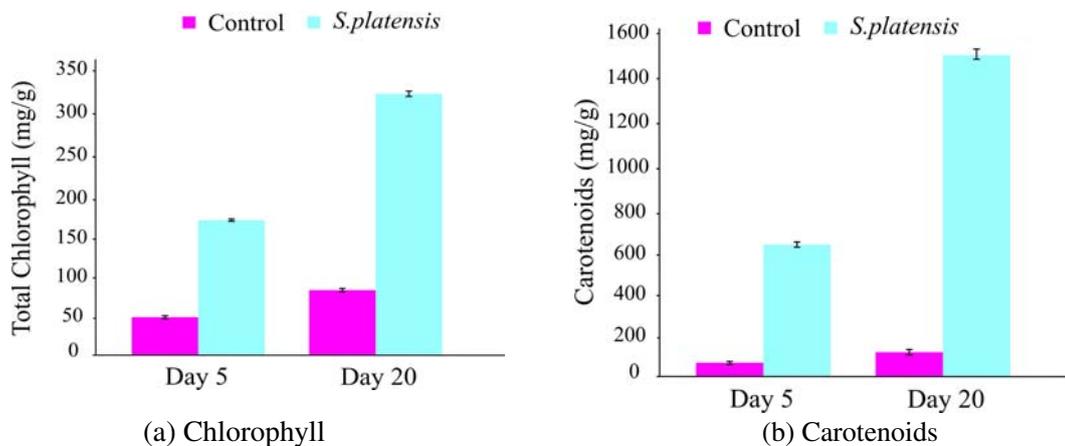
Fig.1 shows the influence of *S. platensis* at various concentrations ( 0.001 %, 0.005 %, 0.01, 0.05 %, 0.1 %, 0.5 %, 1 % and 5 % ) on the germination of *A.dubius*. Seed germination was 82 % in control, 95 % in 0.005 % of *S.platensis* treated plants. At the lower concentration of 0.001 %, the germination was 87 %. As the concentration was increased above 0.01 %, inhibitory effect was observed. This is due to the high concentration of nutrients that osmotically blocked imbibition and absorption of water. Enzymes used to mobilize reserved nutrients in the endosperm are utilized only when the hydrolytic enzymes are activated by water. So reduced availability of water reduced the germination rate. Minerals present in *S. platensis* served as cofactors for majority of hydrolytic enzymes. This accelerated the metabolic rate culminating in increased germination. Synthesis and secretion of IAA in *S.platensis* was reported [13].



**Figure 1. Influence of *S.platensis* on Germination of *A. dubius***

### 3.2 Influence of *S.platensis* on fortification of *A. dubius* leaves

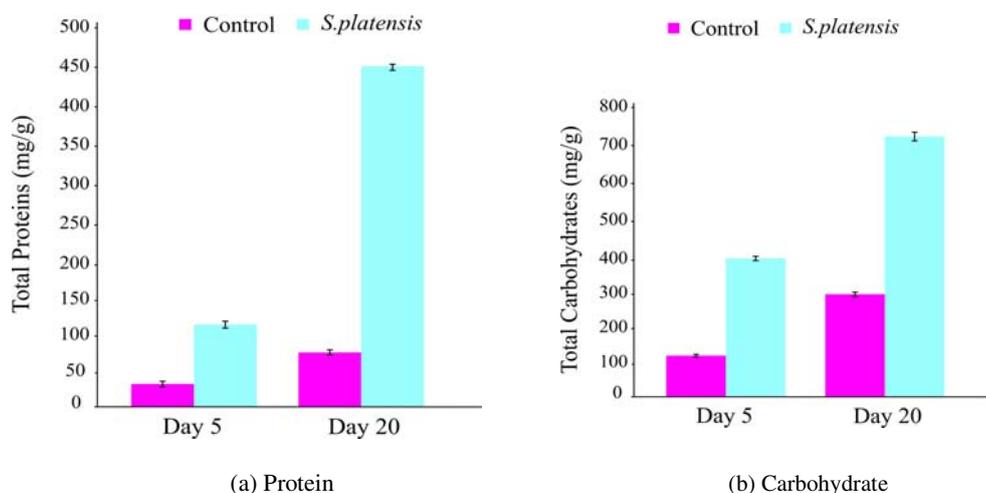
Fig. 2a shows the influence of *S.platensis* on the concentration of total chlorophyll. The concentration of total chlorophyll in leaves on 5th day in control was 49.5 mg/g of dried leaves. In *S. platensis* treated plants the concentration was remarkably increased to 155 mg/g. On 20th day also significant difference between control (85.6 mg/g) and treatment (325 mg/g) was observed. Increased availability of Mg and nitrogen in the soil obtained from *S. platensis* has facilitated the synthesis of chlorophyll in leaves. Porphyrin is the major constituent of chlorophyll. Availability of nitrogen in the soil increases the synthesis of protoporphyrin and then into chlorophyll. Association between the soil nutrients and the concentration of photosynthetic pigments, photosynthetic rate, yield characteristics and nutritional profile of the yield were reported [14].



**Figure 2. Influence of *S. platensis* on Chlorophyll and Carotenoids on *A. dubius***

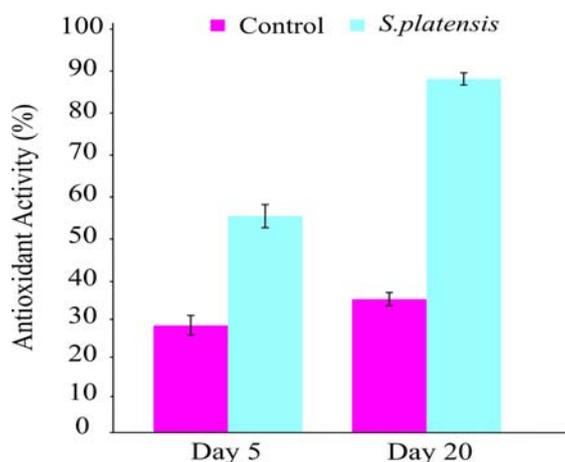
Fig. 2b shows the influence of *S. platensis* on the concentration of carotenoids in leaves. The concentration of carotenoids on 5<sup>th</sup> and 20<sup>th</sup> day were 71.85 mg/g and 118.9 mg/g respectively. In biofortified leaves, it was significantly increased to 650 mg/g and 1500 mg/g on 5<sup>th</sup> and 20<sup>th</sup> day respectively.

Fig. 3a shows the influence of *S. platensis* on the concentration of proteins in leaves. The concentration of proteins on 5<sup>th</sup> and 20<sup>th</sup> day was 31.5 mg/g and 71.93 mg/g respectively. In biofortified leaves, it was significantly increased to 115 mg/g and 450 mg/g on 5<sup>th</sup> and 20<sup>th</sup> day respectively. Increased availability of nitrogen has increased the synthesis of proteins [14]. Proteins are indispensable for the structure and function of cells. Increase in the concentration of proteins indicates the positive impact of biofortification. Influence of organic farming in increasing the concentration of carotenoids and phenolic compounds were well studied [15]. Fig. 3b shows the influence of *S. platensis* on the concentration of carbohydrates in leaves. The concentration of carbohydrate on 5<sup>th</sup> and 20<sup>th</sup> day was 107.2 mg/g and 299.6 mg/g respectively. In biofortified leaves, it was significantly increased to 405.3 mg/g and 723.98 mg/g on 5<sup>th</sup> and 20<sup>th</sup> day respectively. Increase in the expression of starch biosynthesis enzymes like adenosine diphosphate-glucose pyrophosphorylase, starch synthase and starch branching enzyme after the application of organic fertilizer is observed by Song et.al [16].



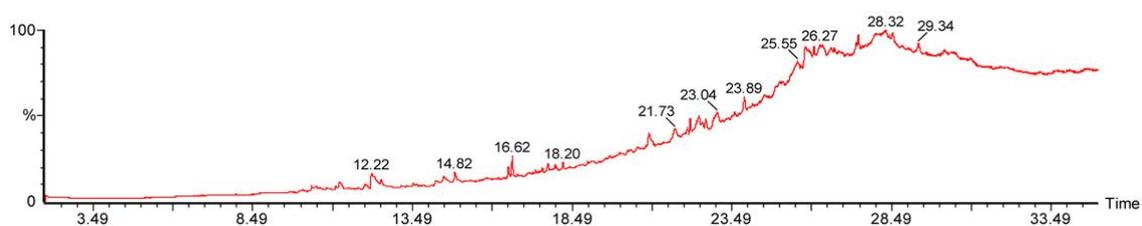
**Figure 3. Influence of *S. platensis* on Protein and Carbohydrate of *A. dubius***

Fig 4 represents the antioxidant activity of *A.dubius* leaves. Antioxidant activity on 5<sup>th</sup> and 20<sup>th</sup> day was 28.45 % and 55 % in control respectively. In biofortified leaves, it was significantly increased to 55 % and 87% on 5<sup>th</sup> and 20<sup>th</sup> day respectively. Carotenoids are well known for their antioxidant activity. Fortification of leaves with *S.platensis* has increased the concentration of carotenoids on 5<sup>th</sup> and 20<sup>th</sup> day. Polyphenolic constituents in *A. dubius* and their antioxidant characteristics was studied by Moyo et.al [17]. All are collectively responsible for the significant increase in the antioxidant activity of fortified leaves.

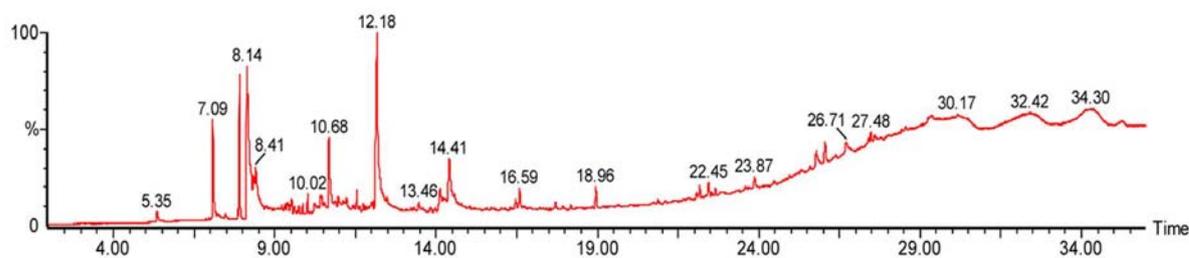


**Figure 4. Antioxidant activity of *A.dubius* leaves**

Fig. 5 and table 1 depicts the GC-MS profile of *A.dubius* leaves (control). It showed the presence of cholestane 3-ol, heptatriacontanol, hexadecanoic acid, etc. Fig 6. and table 2 depicts the GC-MS profile of fortified *A.dubius* leaves. Fortification of leaves with vit A aldehyde is confirmed by the GC-MS spectrum. In addition, it showed the presence of thunbergol, 2H-Pyran 2-(7-heptadecynoxy) tetrahydro, octadecanoic acid, hexadecanoic acid etc. Presence of thunbergol a diterpene in leaves was reported by Adams [18]. Presence of stearic acid in *S.platensis* was reported earlier. Stearic acid and its analogue are potent antidepressants and possess antimicrobial activity [19]. The role of hexadecanoic acid in the structure and function of cell is described well [20]. Vitamin A is important in preventing night blindness. It plays a key role in phototransduction. It plays a significant role in improving the immune system. Fortification in economically inexpensive foods that are consumed in good quantities are a viable solution to avoid vitamin A deficiency [21].



**Figure 5. GC-MS profile of *A.dubius* leaves (control)**



**Figure 6. GC-MS of profile *A.dubius* leaves (fortified)**

**Table 1. GC-MS of *A.dubius* leaves (control)**

No.	RT	Name of the compound	Molecular Formulae	MW	Peak Area %
1.	10.33	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	C <sub>14</sub> H <sub>24</sub> O	208	0.23
2.	11.20	Farnesol isomer a	C <sub>15</sub> H <sub>26</sub> O	222	0.54
3.	12.22	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	1.54
4.	14.82	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	C <sub>15</sub> H <sub>26</sub> O	222	0.59
5.	16.62	á-D-Mannofuranoside, farnesyl-	C <sub>21</sub> H <sub>36</sub> O <sub>6</sub>	384	0.49
6.	18.20	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15- tetramethyl-, (E,E)-	C <sub>20</sub> H <sub>34</sub> O	290	0.19
7.	20.90	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15- tetramethyl-, acetate, (E,E,E)-	C <sub>22</sub> H <sub>36</sub> O <sub>2</sub>	332	0.89
8.	22.11	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16- tetramethyl-, (E,E,E)-	C <sub>20</sub> H <sub>34</sub> O	290	0.33
9.	22.46	Squalene	C <sub>30</sub> H <sub>50</sub>	410	0.78
10.	23.04	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9- trimethyl-12-(1-methylethyl)-	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>	306	1.04
11.	23.89	cis-Z-à-Bisabolene epoxide	C <sub>15</sub> H <sub>24</sub> O	220	0.42
12.	25.55	1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O	536	9.16
13.	26.27	2,2-Dimethyl-3-(3,7,16,20-tetramethyl- heneicosa-3,7,11,15,19-pentaenyl)-oxirane	C <sub>29</sub> H <sub>48</sub> O	412	9.15
14.	27.46	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl- heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	C <sub>30</sub> H <sub>52</sub> O	428	12.02
15.	28.32	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C <sub>28</sub> H <sub>48</sub> O	400	26.95
16.	29.34	9,19-Cycloergost-24(28)-en-3-ol, 4,14- dimethyl-, acetate, (3á,4à,5à)-	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468	12.82
17.	30.16	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336	22.85

**Table 2. GC-MS of *A.dubius* leaves (fortified)**

No.	RT	Name of the compound	Molecular Formulae	MW	Peak Area %
1.	5.35	Furan, tetrahydro-2,2,4,4-tetramethyl-	C <sub>8</sub> H <sub>16</sub> O	128	0.61
2.	7.09	Phenol, 2,4-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206	4.03
3.	7.91	D-Mannitol, 1,2:3,4:5,6-tris-O-(1- methylethylidene)-	C <sub>15</sub> H <sub>26</sub> O <sub>6</sub>	302	3.48
4.	8.14	Diethyl Phthalate	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	222	14.53
5.	10.68	Z,Z-10,12-Hexadecadienal	C <sub>16</sub> H <sub>28</sub> O	236	4.37
6.	11.54	Cyclopentaneundecanoic acid, methyl ester	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268	0.58
7.	12.18	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	16.03
8.	13.46	13,16-Octadecadiynoic acid, methyl ester	C <sub>19</sub> H <sub>30</sub> O <sub>2</sub>	290	0.47
9.	14.13	9,12-Octadecadienoic acid (Z,Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	1.83
10.	14.41	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	5.74
11.	16.59	á-D-Mannofuranoside, farnesyl-	C <sub>21</sub> H <sub>36</sub> O <sub>6</sub>	384	0.89
12.	17.71	4,8,12,16-Octadecatetraen-1-ol, 4,9,13,17- tetramethyl-	C <sub>22</sub> H <sub>38</sub> O	318	0.44
13.	18.96	Benzene, 1-fluoro-4-nitro-2-(5- nitrofurfurylidenamino)-	C <sub>11</sub> H <sub>6</sub> FN <sub>3</sub> O <sub>5</sub>	279	0.94
14.	22.45	Squalene	C <sub>30</sub> H <sub>50</sub>	410	0.63
15.	23.87	cis-Z-á-Bisabolene epoxide	C <sub>15</sub> H <sub>24</sub> O	220	0.74
16.	25.79	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8- octahydro-1,1,4a,7-tetramethyl-, cis-	C <sub>15</sub> H <sub>26</sub> O	222	1.49
17.	26.06	Vitamin A aldehyde	C <sub>20</sub> H <sub>28</sub> O	284	1.27
18.	26.71	Aromadendrene oxide-(1)	C <sub>15</sub> H <sub>24</sub> O	220	1.81
19.	27.48	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C <sub>28</sub> H <sub>48</sub> O	400	0.47
20.	30.17	2H-Pyran, 2-(7-heptadecyloxy)tetrahydro-	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336	17.82
21.	32.42	Thunbergol	C <sub>20</sub> H <sub>34</sub> O	290	21.82

## CONCLUSION

The preliminary study proved that *S.platensis* can be used as a fertilizer to augment plant growth and metabolism. Simultaneously it can be used as an agronomic fortifying agent. Economically inexpensive leafy vegetables can be fortified with the essential macronutrients, micronutrients and Vitamin A. An elaborate study is required to understand the mechanism of fortification in a large scale field study.

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