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Research Article

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Effects of Egg Shell Waste and Algae Enrichment on Tomato Plant Nutrition In the Controlled Environment

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Abstract
Egg shell wastes from the egg processing industry, have been considered for enrichment of soil plant nutrition and for contribution to environmental safety by the waste treatment. Egg Shell functions as a good calcium and mineral source and it was an aim to increase Ca content of tomato. Biological fertilizers need to be considered as alternative to chemical fertilizers, because of human and environmental health aspects. There are publications on the use of bacteria and microalgae enriched biological fertilizers, that state biological fertilizers can compete with the chemical fertilizers. We collected the egg shell wastes with students in this project. We had a culture of Chlorella Vulgaris algae for biodiesel production in our laboratory and aimed to use this biomass for plant nutrition, too. Algae enrichment besides eggshell waste addition to soil has not been explored for tomato plant nutrition. Therefore, it was an aim to use the C. Vulgaris algae, as mineral enrichment besides eggshell waste as plant nutrition for tomato in the controlled atmosphere of the laboratory, The treatments were determined as ESW added, algae added and ESW plus algae added to soil compared with blank (no addition). The results showed that ESW + algae combination treatment had increased Ca content in tomato, compared with other treatments as well as B, Fe, Mg, Na, P minerals. Algae addition compared to blank, did not have a contribution in final tomato wet weight, Probably, algae in soil needed nutrients and competed with soil microorganisms for nutrients, and causing adverse effects on growth.

Keywords: Algae, Egg Shell Waste (Esw), Soil, Tomato

Introduction
The processing of eggs in industry has been increased, for production of liquid, frozen or dried powdered eggs. But still discarded egg shell wastes (ESW) are seen as garbage. Egg production worldwide was approximately 80 million tons in 2017 according to statistics, which increased from 62 million tons (FAO, 2004). Egg shell constitutes 11% of total weight of eggs, therefore 8.8 millions of egg shells are approximately discarded as waste. ESW should be sterilized at 80 degrees and then crushed as powder before being discarded, to be considered as environmentally safe. The powdered egg shells obtained in this manner are usually used as animal feed.

The application of egg shells as a neutralizer of acidity in acidic soils, as calcium supplement in calcium deprived soils, as a structure material to enhance the quality of biomaterials are known [1]. Egg shells added to organic wastes as a supplement through composting was studied as potential market for calcium rich commercial compost [2]. ESW use in contaminated soils to stabilize certain heavy metals was published [3]. and function as low-cost adsorbent material for metals in aqueous solutions [4]. and various applications of eggshells as adsorbents were summarized [5, 6]. stated the growth in last ten years in a review paper, such as in removal of toxic heavy metal ions and hazardous dyes. Application of ESW besides rapeseed residue as fertilizer on soil was studied by [7].

One medium sized egg contains approximately 750-800 mg of calcium and a variety of other microelements [8]. Dried and powdered egg shells are a rich source of calcium and protein [9]. Besides calcium in egg shell found as $CaCO3$ other minerals such as phosphorous and potassium are important ingredients of fertilizers. Calcium depriviation causes illness in tomato plant known as Fusarium oxysporum infection and it is seen as rotten or discoloration in fruit surface. The cell wall integrity of plant enriched with calcium is important for plant health [10]. due to increased uptake of calcium in plants leads to decrease in pathogen infection.

The increased use of biological fertilizers increases crop quality and decreases adverse effects such as illnesses in plants [11]. reported the rural development and environmental protection through the use of biofertilizers. The improved plant growth, fruit yield and nutrition was studied by [12]. The increased importance of sustainability of biological systems leads to evaluation of organic agricultural wastes and adding value to food industry. The application of organic fertilizers contributes to soil physical (e.g. structure development), biological (e.g. microflora) and chemical (nutritional) values. The increased long-term biological matter crop quality and soil attributes, such as porous structure and water holding are expected.

Objectives

The main purpose of this research was to utilize egg shell waste (ESW) and algae C.vulgaris in soil plant nutrition. The ESW, algae and ESW plus algae treatment effects were to be investigated in terms of bioavailability to tomato plantlets. The purpose of the research in broader sense, was to use biological fertilizers in order to reduce the use of chemical fertilizers, for increased sustainability and human health.and environment protection.

Materials and Methods

The Tomato Seedlings

The tomato seedlings were obtained from a local producer growing organic 'stick' tomatoes. The organic medium was a liquid medium (brand name Biovin™) specially formulated for tomato production and used in greenhouses. The medium ingredients were total organic material 45%, total organic carbon 19,5%, total Nitrogen 3% and dissolvable potassium as K_2O. Liquid media was formulated (according to the formula provided by the supplier) such that 40 ml of solution dissolved in 20 litres of water. This solution was added to soil tanks (about 10 liters each) at the beginning as nutrients besides water. Later, the soils were watered every two weeks with tap water.

Algae

The algae Chlorella vulgaris added to soil was grown in our laboratory from a strain supplied from a U.S. Biotechnology company. It was suggested for high mineral and oil content for use especially in biodiesel production. This culture was kept in solid TAP medium [13]. as a stock culture and inoculated to liquid medium afterwards. The liquid culture of algae was kept growing under set conditions at 23 C on a shaker oscillating @110 rpm. Later, certain amount was added to soil as supplement. Algae and Egg shell waste (ESW) addition to soil, as explained before, was repeated after one month.

Eggshell Waste (ESW)

Egg shell wastes were collected by the students from consumed cooked eggs in dormitory cafeterias. They were cleaned from ESW membranes and dirts if any, and the put in the oven at 105 C for sterilization of the shells. The shells turned to brownish color in the oven after about half hour. Afterwards they were crushed in kitchen type blender and ESW powder was obtained.

The $CaCO_3$ in egg shell should be converted to calcium which is available form for plant uptake. The acetic acid (Technical grade) is used for the purpose of Ca exposure for plants. ESW powder was put in 3% acetic acid solution in a beaker enough to cover the surface and left overnight for exposure to acid. The following day, the acid was poured from the beaker leaving the powder and washed with water.

Plant Growth Room

The plant growth room had temperature and humidity controls and kept at a temperature of 24-25 C and approximately 43-45% humidity. LED lights (OSRAM LED Tubes 21W/865 ST8P-1,5 M) were specially constructed on the shelf where plants were located. The soil filled pots (1,5 m length rectangular) were placed on the shelf under the lights in the growth room. The growth of tomato plantlets in the soil pots in the growth room is shown in Picture 1.

quality and nutrient uptake of A. Andreanum. Scientia Horticulturae 125: 434-441.

Picture 1. The growth of tomato plantlets in soil in the plant growth room.

Results

The experimental design for the experiments were conducted such that:

Soil and pots were weighed. Weight of soil going to each pot besides the weights of pots were recorded. The soil pots were based on four treatments:

T1.Soil + algae (Algae)
T2. Soil + eggshell waste powder (ESW)
T3. Soil + eggshell waste (ESW+ Algae)
T4. Soil only (Blank)
(ESW, Algae, (ESW+Algae) and Blank abbreviations will be used lateron)

The tomato plantlets, 4 for each pot, were weighed at the beginning and almost the same amount (total weight of 4 plantlets going to each pot) were achieved. The eggshell powder (as explained in materials) drained & weighed (15.4 g for one teaspoonful measure). From this ESW powder, 1 teaspoonful was used for each plantlets i.e. 4 tsps put in each tank for 4 plantlets, The algae grown (as explained in materials) in 1 L flasks on the shaker were used to add to pots such that 500 mL of liquid algae culture was put in the soil pot. Blank treatment had soil with hydroponic only (no additions).

Experiment was terminated after growth and harvesting of fruits was achieved (2 months period).

After harvesting of fruits, weights of tomatoes were recorded for each treatment (Table 1). Mineral analysis for the plant stalk and tomato were done by ICP Instrument. The model of the instrument was ICP Perkin Elmer Optima ICPOES 7000DV. The analysis of minerals B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, Zn were done for the stalk and tomato. Table 2 and Table 3 shows the results of mineral analysis for the stalk and tomato.

Results are given in the following tables and figures:

Table1: Final wet weight of harvested tomatoes.

	Tomato wt (g)
Algae	106.16
ESW	241.45
ESW +algae	25.48
Blank	254.20

Table2: Mineral analysis results for tomato (ppm).

	B	Ca	Cu	Fe	K	Mg	Mn	Mo	Na	P	Zn
ESW	20.5	966.25	5	3.25	26.42	1280	8.75	0.62	1792.5	3052.5	22,5
ESW +algae	20.62	1776.25	4.5	22.87	26.95	1278.75	9.75	0.75	1568.75	2922.5	20.62
Algae	20.62	1426.25	6.75	25.25	26.25	1385	8.75	0.87	1475	2768.8	25.25
Blank	18.5	1531.25	4.5	20.87	28.51	1234.87	9.12	0.87	1547.5	2813.8	20.5

Table3: Mineral analysis results for plant stalk (ppm).

	B	Ca	Cu	Fe	K	Mg	Mn	Mo	Na	P	Zn
ESW	1.45	5771.25	3.25	21.5	22.67	1582.5	11.25	0.87	6805	1268.8	31.37
ESW + algae	14.25	4662.5	3.5	19.62	23.41	1591.25	116.25	1	7298.75	1235.5	33
Algae	14.5	5525	4.75	22.75	22.28	1437.5	13.12	0.87	6721.25	1135.3	35.5
Blank	14.25	5571.25	2.87	18	22.67	1456.25	13	0.87	6711.25	1081.4	34.37

Because our major aim was to add to calcium content in tomato in order to avoid diseases, calcium content in tomato was in major scope of this research. The mineral analysis of tomato results showed that the combination of ESW and algae treatment increased Ca content, which was seen as a positive effect. ESW + algae treatment gave increased Ca, B, Fe, Mg, Na, and P results, too. ESW only gave increased B, Mg, P, Zn results. Algae only gave B, Cu, Fe, Mg and Zn results. Mineral analysis for tomato (fruit) are shown in Figure 1 (data given in Table 2). Results show that both ESW+ algae combination contributed to calcium content positively and gave higher amounts than the control (Table3). Most minerals in tomato were found higher for ESW + algae added treatments.

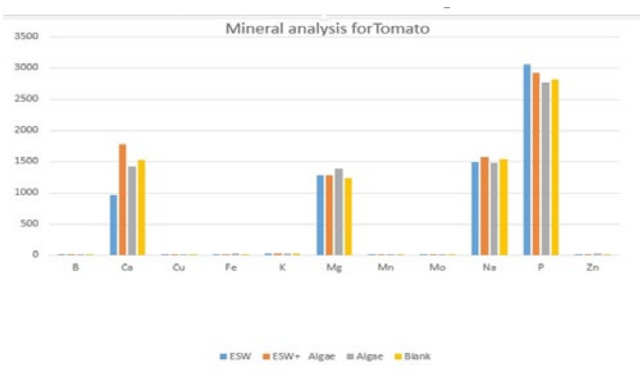

Figure1. Mineral analysis results for different treatments for tomato

Figure 1

The results were evaluated in terms of mineral content in the stalk and tomato. The results of mineral analysis for plant stalk are shown in Figure 2 (data given in Table3). Treatment effects were evaluated in terms of most contribution for mineral contents. The combination of ESW and algae addition seemed to increase Fe, K, Mg, Mn, Mo, Na, P minerals. ESW treatment had increased Ca, Fe, Mg, Na, P contents of stalk. ESW increased Ca content individually, but Algae treatment, and ESW+ algae treatments did not increase Ca content in stalk.

Abbreviations used in tables & figures: Algae; Algae added treatment ESW; Egg shell waste added treatment, ESW + algae; Egg shell waste plus algae added treatment, Blank; no addition

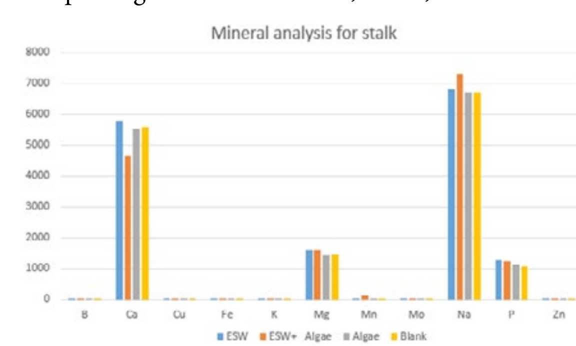

Fig2. Mineral analysis for different treatments on stalk

Figure 2

Conclusion and discussion

All treatments had growth of plants and gave number of ripened tomatoes to be harvested. The results of final wet weight of harvested tomatoes (Table1) showed that the highest weight was obtained for blank (no addition). This result may mean no treatment of the research had a positive effect on tomato weight increase. Algae addition to soil seemed to have an adverse effect on the growth of tomatoes, probably because alage as living organisms require nutrients from the soil, they may be in competition with soil microorganisms.

The ESW + Algae combination treatment in soil was found to be beneficial for tomato mineral enrichment especially leading to increased calcium content in tomato. It can be concluded that increased calcium and some other minerals e.g. B, Fe, Mg, Na, P minerals in tomato can be expected so it will be beneficial for the grower. Benefits will come to environment protection and human health too, based on the controlled environment in plant growth rooms or greenhouses. Clean room free from insects, pesticides contaminants etc. and controlled temperature and humidity conditions, medium constitiuents are the advantages of controlled environment study. Algae grown under controlled conditions is seen as clean and sustainable source and it is safe. But, algae in soil seems to compete for nutrients with the soil indigeneous organisms and thay may have adverse effect as seen by decreased tomato weight of the harvested fruits. Soil has complex microflora itself e.g. soil nitrogen fixation bacteria etc. existence require soil nutrients and algae may come as competiton with other organisms, leading to decreased weight of fruit production.

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