



Role of Azolla Pinnata Biofertilizer Extract in Producing Healthy Tomatoes

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2018/v3i329832

Editor(s):

(1) Dr. Khadiga Mohamed Abu-Zied, Professor, Department of Photochemistry, National Research Centre, Cairo, Egypt.

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Reviewers:

(1) Paul Kweku Tandoh, Kwame Nkrumah University of Science and Technology, Ghana.

(2) Ibrahim Yerima, University of Maiduguri, Nigeria.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/46785>

Original Research Article

Received 25 October 2018

Accepted 15 January 2019

Published 17 January 2019

ABSTRACT

Well-known commercial Tomato seeds (Alisa) were obtained from a famous local store. All seeds were grouped into 7 groups (5%, 10%, 20%, 30%, 40%, 50%, and Control), which were equivalent to different Azolla biofertilizers extract. Seeds of each group were left immersed in the equivalent concentration 24 hours before sown in pots with growing media of 1 peat: 1Vermeculite at the beginning of summer season (First week of May). At the time of transplantation to the field, three pots of each group were used for seed germination test; Seeds were transplanted into the field after 6 weeks. After three days of transplantation, control solution (without Azolla biofertilizer) and different Azolla biofertilizers were foliar applied, the process was repeated every 15 days till 45 days after transplanting. Randomized Complete Block Design with three replicates was adapted. Each block consisted of two rows of 1.5m in wide and 5 m long (15 m²/plot), plant spacing was 50 cm, and each replicate has 20 plants. The suggested development approaches for the summer season were conducted all through the developing season. Vegetative growth of the tomatoes was expressed as plant height, plant length, branch flower clusters, and fruits were estimated in five plants/plot after 50 days from the date of transplanting. Tomatoes of control group and Tomatoes that showed a strong vegetative growth were used for feeding Winstar rats. All Biochemical parameters showed a highly significant difference compared to the control group.

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Keywords: *Azolla pinnata*; tomatoes; *Azolla* biofertilizer extract; foliar application; biochemical parameters.

1. INTRODUCTION

We live on a planet in which the continuous rising human population together with a decrease in all-natural resources are definitely the major reasons for environmental damage around us. The need for improving plants production has become an immediate want to save our everyday life. Chemical fertilizers have resulted in polluting the environment along with groundwater, in addition to finding trapped in harvest crops and moved to our systems. Biofertilizers are eco-friendly fertilizers, which used to boost the quality and soil fertility. Bio-fertilizers are made of natural waste items and they don't include any chemical compounds. They can be good for the land since they enhance the soil with microorganisms which help in providing natural and organic nutrients, which help the soil to avoid diseases. They consequently improve the nutritious quality of the soil, additionally, they restore the depleted nutrients of the soil. There are many studies revealed the beneficial features of biofertilizers with many crops, which include beans and cereals [1] vegetables [1,2,3,4]; the majority of these studies confirmed the positive effects on plant growth, on the other hand, there were a few studies examined composts are broken up. Because of the rising demand for natural plants, Scientists working on new choices to traditional fertilizers that resulting in some health and fitness concerns; biofertilizers are typically living microorganisms used to replace the chemical like fertilizers [2].

Green manure is definitely the technique of transforming of natural harvest towards soil probably by supplying them in similar area or vegetation developed another place within the natural stage just before launching flowers and integrated into the garden soil [5]. The variety of green fertilizer plants exist in the world, which plays a role in the garden soil nutrients characteristics and regains garden soil overall health. It is now noticeable the green fertilizer herbs not just boost garden soil overall health and fertility it also helps with control farming pets [6].

There are almost 200 million tons each year required from Nitrogen for agricultural purposes to produce human's food as well animals and industrial foods; this is what makes Nitrogen the most important factor in the agricultural system.

It's well-known that the atmosphere has about 80% Dinitrogen gas (N₂) but the plants can't get any direct benefit of this gas, therefore there should be a process where the plants can get dinitrogen gas; this can be easily done using microorganisms through Nitrogenase enzyme to convert N₂ into Ammonia (N₃) which in turn can be added to the plants in form of organic components; this is what is so-called Biological N₂ fixation.

Azolla is a genus of 5 to 8 varieties of suspended marine plants that previously belongs to family Salvinaceae [7], while modern researches put *Azolla* in family Azollaceae [8] which is a family of heterosporous ferns in the order Salviniales [9]. *Azolla* also was known as Mosquito ferns or Duckweed that create a mutual partnership with cyanobacteria with the ability to fix Nitrogen [10,11,12]. It multiplies sexually and asexually by dividing. It could double its biomass within just about three to ten days according to surrounding conditions, maximum *Azolla* yield can achieve around 9 Tons of fresh new biomass/ha.

Azolla has a wonderful track record as green manure in most countries across the world [13,14]. The great advantage of *Azolla* is certainly not only is due to a top quality feed for animals along with a great choice for contributing to compost as well as backyard plants, however that it develops alone without having to affect fertility in the system. It is a bit more like cost-free power and extremely regenerative like a system ingredient.

Azolla pinnata has substantial nitrogen content and has been utilized as an environmentally friendly fertilizer for wetland rice farming as well as for their capability to preserve considerable amounts of nutrients [15,16]. It is amongst the additional types of fertilizer utilized in addition along with other bio-fertilizers. Vegetable production is affected by correctly grown transplants. Transplants high quality is extremely reliant from numerous things which include lamination, temperatures, Carbon dioxide, and air humidity, the supply of water, fertilizing, substrate, growing techniques, veggie varieties or types [17-26].

Tomato (*Lycopersicon esculentum* Mill) is a member of family Solanaceae and is a standout amongst the most broadly eaten vegetables in

the world which famously comes from the way that they can be eaten alone or as an ingredient in numerous recipes. Tomato is among the most important vegetables on this planet, it is one of the most widely cultivated vegetable crops in the world [27,28]. It got their start in Latin America, Due to its importance as food items, tomato could possibly be carefully bred to boost output, fresh fruit quality, and possibilities to manage biotic and abiotic challenges. Tomato is placed in a first place on all vegetables and fruit as a supplier of nutritional supplements in the U.S. [29]. Tomato represents an important function in the human being a healthy eating plan. It really is full of phosphorus, iron and vitamin A, B and C. [30,31,32].

For many continual decades, the utilization of tomatoes has been related with aversion of a few maladies [33,34], due to the substance of cancer prevention agents (antioxidants) including carotenes, (Lycopene as well as β -carotene), ascorbic acid phenolic compounds [35].

As a plant it makes up an essential ingredient in person's eating plan, particularly developing nations around the world. On the other hand, each household intake of fresh vegetables in the western world is commonly greater than in developing countries, probably because individuals in the western world possess a much better understanding of the nutrients of vegetable herbs [28,36].

Tomato is indigenous to warm South and Central America, in which it had become grown in pre – Columbian periods. Its wild progenitor is believed to have been the cherry tomato, *L. esculentum* var. *cerasiforme*, which develops wild within the Peru – Ecuador location even though tomato plants had been most likely domesticated from weedy types which in fact had distributed so far as north Mexico [31,37].

Tomatoes exhibit a large climate tolerance and could be cultivated in outdoors anywhere there may be greater than ninety days of snow totally free weather conditions. Tomato is much more productive where you can find prolonged warm times. The ideal growing climate is 21°C to 24°C. At these temperatures, top quality seeds are going to take about one week to come out. Temperature influence blooming and pollination. If climate is under 15°C or higher 29°C, plant pollen launch is limited leading to partial feeding of ovules. This will cause flattened fresh fruit surfaces and development of serious indent

inside the fruits, a phenomenon known as cat face [28,38].

2. MATERIALS AND METHODS

2.1 Strain and Growth Conditions

2.1.1 Strain: *Azolla Pinnata*

Growth Conditions: *Azolla pinnata* strain was grown and purified several times in Yoshida medium [39]. 10 grams *Azolla pinnata* had been developed in plastic planting pots (32 centimeters in diam. and 15 centimeters dep.), every pot including 1 Kilogram garden soil in 3 liters plain tap water then stored in a garden greenhouse until *Azolla* coated the whole water area. *Azolla* was accumulated and involved in .01 mercuric chloride for 1 minute, rinsed carefully in running regular water for many moments, by using a screen of .2 fine mesh, after which air dried out on tissue paper for 30 minutes. The obtained fronds were utilized as a possible inoculum for more findings [40].

Preparation of *Azolla* biofertilizers extract: About one Kg of *Azolla* was boiled in 1 Liter distilled water for about 30-45 minutes, after filtration, filtrate was considered as 100% raw *Azolla* extract, from which different concentrations were made (5%, 10%, 20%, 30%, 40%, and 50%) by means of distilled water. Control solutions without *Azolla* biofertilizers extract) was prepared. All *Azolla* biofertilizers and control solutions were kept refrigerated prior to use [41].

Tomatoes Seeds: Well-known commercial Tomato seeds (*Alisa*) were obtained from a famous local store. All seeds were grouped into 7 groups (5%, 10%, 20%, 30%, 40%, 50% and Control), which were equivalent to different *Azolla* biofertilizers extract. Seeds of each group were left immersed in the equivalent concentration 24 hours before sown in pots with growing media of 1 peat: 1Vermiculite at the beginning of summer season (First week of May). At the time of transplantation to the field, three pots of each group were used for seed germination test, Seeds were transplanted into the field after 6 weeks. After three days of transplantation, control solution (without *Azolla* biofertilizer) and different *Azolla* biofertilizers are foliar applied, the process was repeated every 15 days till 45 days after transplanting. Randomized Complete Block Design with three replicates was adapted. Each block consisted of two rows of

1.5m in wide and 5 m long (15 m²/plot), plant spacing was 50 cm, and each replicate has 20 plants. The suggested development approaches for the summer season were conducted all through the developing season [42]. Vegetative growth of the resulted tomatoes expressed as plant height, plant length, branch flower clusters, and fruits were estimated in five plants/plot after 50 days from the date of transplantation. Tomatoes of control group and Tomatoes that showed strong vegetative growth (plant length, plant height, number of leaves and branch clusters) were used for feeding Wistar rats.

Preparing Tomatoes for Rats Feeding:

Tomatoes of the control group and Tomatoes that showed strong vegetative growth were peeled and cut into tiny parts and introduced to three groups of rats as a sole recipe once daily in the ratio of 100, 500 and 1000mg/kg. Rats don't allow to eat any other food throughout the entire experiment other than drinking water. Rats of the control group fed on commercial rat food and drinking water.

Wistar Rats: Twenty Wistar rats (males), weighing about 180 gram each, were randomly divided into four groups; each of five individuals, they allowed 15 days to acclimatize with the new environment after which the experiments started for six weeks.

Blood Collection: Blood was collected from rats after every two weeks, obtained serum was kept refrigerated until it was used.

Estimation of blood biochemical Parameters:

This work was designed to test the following parameters: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) analyses were done according to Reitman-Frankel method [43], Cholesterol was done according to Kulkarni [44], Triglycerides was done by using the Abbott ARCHITECT C-8000 system [45] and were

compared with the University of Alabama School of Medicine (Birmingham, Alabama) laboratory (n = 40, r = 0.997, bias = -0.05%). Overall, the analytical performance of lipid measurements met guideline-established benchmarks and are further detailed [46], total proteins was done according to Henry et al. [47], Creatinine analysis was done according to Henry [48] and the uric acid analysis was done according to Young [49].

2.2 Statistical Analysis

Data for Effect of Tomato Grown in 20% Azolla Biofertilizer Extract on Biochemical Parameters were subjected to analysis of variance by the least significant differences (LSD) [50].

3. RESULTS AND DISCUSSION

Data in Table (1) showed that amongst all Azolla fertilizer extracts, 20% concentration showed the maximum increase in germination, Shoot length, Root length, Fresh and Dry weights compared to control and other Azolla biofertilizers extract Concentrations (97%, 20.00 cm, 8.2 cm, 4.2 g, and 0.890 g, respectively; after which all parameters decreased; this may be due to the presence of few natural growths promoting hormones such as Cytokinins, auxins, vitamins and amino acids in the lower concentration which encourage both seeds' germination and plants growths. These findings were in full agreement with that obtained by Bindhu [51].

Data in Table (2) showed that all vegetative growths parameters were greatly affected by Azolla biofertilizers extract and foliar application, 20% Azolla biofertilizer concentration expressed the highest effect on all vegetative growth parameters compared to control and other Azolla biofertilizers concentrations, values for vegetative growth parameters were 49.5cm, 8.3, 9.0, 180g, 130 and 50.0 days for plant length, branches number, leaves number, fruit weight, fruit number

Table 1. Effect of Azolla extract on germination and growth of Tomato (*Lycopersicon esculentum* Mill)

Group	Germination%	Shoot Length (Cm)	Root Length(cm)	Fresh Weight(Gm)	Dry Weight(Gm)
Control	81	12	4.5	1.75	0.665
5%	91	15	5.4	1.96	0.735
10%	93	17.3	7.00	2.45	0.840
20%	97	20.00	8.2	4.2	0.890
30%	90	13.8	5.0	1.85	0.703
40%	87	11.5	4.6	1.78	0.676
50%	55	11	3.8	1.76	0.668

and ripening time respectively. For Fruit Characteristics, data revealed that Azolla biofertilizer greatly improved fruit weight, numbers and ripening time which has commercial potential for farmers, these data also reported by Isah et al. [52], who confirmed that green manure has a valuable contribution to the growth and yield of Tomatoes.

Data in Table (3) showed that all biochemical parameters were highly affected in rats fed on tomato grow in 20% Azolla Biofertilizer Extract and Foliar Application compared to the control group that fed on commercial rat food; the maximum difference was shown when rats fed on 1000mg/kg for all biochemical parameters. ALT parameters were 4.85(U/L), 4.57(U/L), and 4.31(U/L) compared to 5.00 (U/L) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively. AST parameters were 13.86(U/L), 13.62(U/L), 13.45(U/L) compared to 14.00(U/L) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively. Creatinine parameters were 0.82(U/L), 0.81(U/L) and 0.78(U/L) compared to 0.85(U/L) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively.

Uric Acid parameters were 2.70(U/L), 2.65(U/L) and 2.55(U/L) compared to 2.50(U/L) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively. Cholesterol parameters Were 4.27(mmol/l), 4.25(mmol/l) and 4.15(mmol/l) compared to 4.30(mmol/l) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively. Triglycerides parameters were 1.73 (mmol/l), 1.69(mmol/l) and 1.65(mmol/l) compared to 1.75(mmol/l) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively.

Total Protein parameters were 50.60(G/l), 50.67(G/l) and 50.68(G/l) compared to 50.38(G/l) of the control group when rats fed on 100mg/kg, 500mg/kg and 1000 mg/kg of tomato grown in 20% Azolla Biofertilizer Extract and Foliar Application respectively. The whole data revealed that tomato grown in 20% Azolla Biofertilizer Extract and Foliar application showed a great effect on all biochemical parameters, this finding could be attributed to the fact that Tomato contains strong antioxidant properties; Lycopene which is a red pigment found in many vegetables

Table 2. Vegetative growth of tomatoes with Azolla biofertilizers extract at fruit set stage

Group	Plant length (Cm)	Branches Number	Leaves Number	Fruit		
				Weight(G)	Number	Ripening time (Days)
Control	44.0	7.00	7.00	125.00	90.00	60.0
5%	46.5	7.5	7.00	127.00	95.00	57.0
10%	47.3	7.7	7.00	128.5	99.00	55.0
20%	49.5	8.3	9.0	180.00	130.00	50.0
30%	48.1	8.1	7.00	145.00	115.00	53.0
40%	45.3	7.2	6.00	133.00	110.00	52.0
50%	45.1	7.1	5.00	130.50	105.00	52.0

Table 3. Effect of tomato grown in 20% Azolla Biofertilizer extract on biochemical parameters

Biochemical Test	ALT (U/L)	AST (U/L)	Creatinine (U/L)	Uric acid (U/L)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Total Proteins (G/l)
Control	5.00	14.00	0.85	2.50	4.30	1.75	50.38
100mg/kg	4.85	13.86	0.82	2.70	4.27	1.73	50.60
500mg/kg	4.57	13.62	0.81	2.65	4.25	1.69	50.67
1000mg/kg	4.31	13.45	0.78	2.55	4.15	1.65	50.68
Significance	**	**	**	**	**	**	**
LSD _{0.01}	0.1289	0.1231	0.04872	0.06890	0.04537	0.04826	0.1462

**= Significant at 0.01 from Control.

and fruits is the most important antioxidant agent present in tomato and considered as the main responsible substance for the antioxidant effects of tomato according to many studies [53,54,55]. In the same way, it's well known that Azolla is recognized by its higher nutrition value, for that reason can replace tradition proteins products [56]. Also, we could conclude that Azolla Pinnata played an important role in maximize the effect of valuable antioxidant properties of tomato due to its ability to provide tomato with its valuable contents of Vitamins, Beta Carotene, Growth Promoters and Minerals which in turns help tomato fruits to have many advantages [57,58].

4. CONCLUSION

Azolla pinnata Biofertilizer Extract used for Tomato growth along with foliar application; the best results were shown with 20% Azolla Biofertilizer Extract, all biochemical parameters tested in this study showed a highly significant difference compared to control group. We plan to have a further study to clarify the transfer mechanism of Azolla beneficial nutrient to tested crops.

ACKNOWLEDGEMENTS

We are grateful to Dr. Reda Mohamed El Shahat Mohamed Prof. Dr. Microbiology, Dep. Soils and Water Environment Research Institute (SWERI), Agric. Research Center, Giza, Egypt. For the great help throughout the entire study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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