Role of Azolla Pinnata Biofertilizer Extract In producing Healthy Tomatoes.

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 are were foliar applied, the process was repeated every 15 days till 45 days after transplanting. Complete randomized blocks 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 resulted tomatoes were 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 transplanting. Plants of Control and Plants showed strong vegetative growth in plant branch length, plant height, number of leaves and branch clusters were used for feeding Winstar rats. All Biochemical parameters showed a highly significant difference compared to the control group.

Keywords: Azolla Pinnata, Tomatoes, Azolla biofertilizer extract, foliar application, Biochemical Parameters

Introduction:

With no Without doubt, we live on a planet in which the continuous rising human population together with a decrease in all-natural resources sources are definitely the major reason for environmental damage we have seen all around us. The need for improving plants production grew to has become an immediate desire to save our everyday life. Chemical fertilizers has resulted into polluting the environment along with groundwater, in addition to finding trapped in harvest crops and moved to our systems. Biofertilizers in the very easy meaning is a number of necessary natural and organic ingredients within a preparation that has survival microorganisms that capable to fix nitrogen. For that reason several studies have demonstrated beneficial features of biofertilizers over a large range of crops, that include dried beans and cereal products, cosmetic, and blooming plants and flowers **[1]**, vegetables **[1,2,3&4]**; Almost all these studies have established that natural garden compost normally has significant positive effects on plant growth. But, there was clearly pretty very few experimental studies examining composts are broken up, peat-like compounds developed using a non-thermophilic approach between biodegradation and placement of organic ingredients by means of connections about worms and microbes.

Because of rising demand on natural plants, Scientists working on new choices to traditional fertilizers that resulting in several 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 Saliviniaceae **[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, 18, 19, 20, 21, 22, 23, 24, 25& 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 proginator is believed to have been the cherry tomato, L. esculenton 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].**

MATERIALS AND METHODS:

Strain and Growth Conditions:

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: Boil about 0ne Kg of Azolla 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: 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 are foliar applied, the process was repeated every 15 days till 45 days after transplanting. Complete randomized blocks 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. Plants of control and Plants showed strong vegetative growth (plant length, plant height, number of leaves and branch clusters) were used for feeding Winstar rats.

Preparing Tomatoes for Rats Feeding: Tomatoes of the control group and 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 **[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]** and Total Proteins was done according to **[47].** Creatinine analysis was done according to **[48]** The uric acid analysis was done according to **[49].**

Statistical analysis:

Data were subjected to analysis of variance by the least significant differences (LSD) **[50].**

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 **[51].**

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

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 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 **[52],** who confirmed that green manure has a valuable contribution to the growth and yield of Tomatoes.

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**

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 13.8(U/L), 13.2(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 2.55(U/L), 2.56(U/L) and 2.70(U/L) compared to 2.5(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 1.74(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 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].

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**	**2.55**	**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**	**2.70**	**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.**

CONCLUSION:

Azolla pinnata Biofertilizer Extract used for Tomato growth along with foliar application; 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.

REFERENCES:

1- Chan, P.L.S. and Griffiths, D.A. 1988. The vermicomposting of pre-treated pig manure, Bioi. Wastes, 24:57-69.

2- Edwards, C.A. and Burrows, I. 1988. The potential of earthworm composts as plant growth media, in *- Edwards, C.A. and Neuhauser, E., Eds., Earthworms in Waste and'Environmental Management, SPB Academic Press, The Hague, the Netherlands, pp. 21-32.

3- - Subler, S., Edwards, C. A., Metzger, J. (1998) Comparing composts and vermicomposts. BioCycle 39, 63–66.

4- Atiyeh, R.M., DomInguez, J., Subler, S., and Edwards, C.A. 2000a. Biochemical changes in cow manure processed by earthworms (Eisenia andrei) and their effects on plant-growth, Pedobiologia, 44:709-724.

5- Pieters, J.A., 1927. Green Manuring, Principles and Practice. John Wiley and Sons, New York, pp: 365.

6- Anon, 1982. Dairy Facts and Figures. Thames Ditton, Surrey: The Federation of United Kingdom Milk Marketing Boards.

7- Metzgar JS, Shneider H, Pryer KM 2007. Phylogeny and divergence time estimates for the fern genus Azolla (Salviniaceae). International Journal of Plant Sciences. 168: 1045-1053.

8- Atousa Farahpour-Haghani, Mahdi Hassanpour, Faramarz Alinia, Gadir Nouri-Ganbalani, Jabraeil Razmjou1, David Agassiz. Water ferns Azolla spp. (Azollaceae) as new host plants for the small China-mark moth, Cataclysta lemnata (Linnaeus, 1758) (Lepidoptera, Crambidae, Acentropinae). Nota Lepi. 40(1) 2017: 1–13.

9- Alan R. Smith, Kathleen M. Pryer, Eric Schuettpelz, Petra Korall, Harald Schneider & Paul G. Wolf (2006). "A classification for extant ferns Taxon. 55 (3): 705–731.

10- PETERS, G. A. & MAYNE, B. C. (1974). The AzollaAnabaena azollae relationship. I. Initial characterization of the association. Plant Physiology 53, 813-819.

11- Peters GA, JC Meeks 1989 The Azolla-Anabaena symbiosis: basic biology. Annu Rev Plant Physiol Plant Mol Biol 40:193–210.

12- Plazinski J, Q Zheng, R Taylor, L Croft, BG Rolfe 1990 DNA probes show genetic variation in cyanobacterial symbionts of the Azolla fern and a closer relationship to free-living Nostoc strains than to free-living Anabaena strains. Appl Environ Microbiol 56: 1263–1270.

13- Lumpkin, T.A., Plucknett, D.L., 1982. Azolla as a Green Manure: Use and Management in Crop Production. Westview Tropical Agriculture Series, No. 5. Westview Press, Bolder, CO.

14- Bhuvaneshwari K and Ajay Kumar. Agronomic potential of the association Azolla – Anabaena. Science Research Reporter 3(1):78-82, April 2013.

15- Arora, A. and Singh P. K. Comparison of biomass productivity and nitrogen fixing potential of Azolla sp. Biomass Bioenerg.,24: 175-178 (2002).

16- Kumar, S. R. S. and Rao, K.V. B. Biological nitrogen fixation: A Review. Int J Adv Life Sci., 1:1-6 (2012).

17- Atherton J.G., Rudich J., 1986. The tomato crop. A scientific basis for improvement, London, New York.

18- Weston L.A., 1988. Effect of flat cell size, transplant age and production site on growth and yield of pepper transplants. HortScience, 23 (4), 709–711.

19- Ciardi J.A., Vavrina C.S., Orzolek M.D., 1998. Evaluation of tomato transplant production methods for improving establishment rates, HortScience, 33 (2), 229–232.

20- Vavrina C.S., 1998. Transplant age in vegetable crops. HortTechnology, 8 (4), 550–555.

21- Damato G., Trotta L., 2000. Cell shape, transplant age, cultivars and yield in broccoli. Acta Horticulturae, 533, 145–152.

22- Głowacka B., 2002. Wpływ barwy światła na wzrost rozsady pomidora (Lycopersicon esculentum Mill.). Acta Sci. Pol. Hortorum Cultus, 1 (2), 93–103.

23- Paul L.C., Metzger J.D., 2005. Impact of vermicompost on vegetable transplant quality. HortScience, 40 (7), 2020–2023.

24- Brazaitytė A., Duchovskis P., Urbonavičiūtė A., Samuolienė G., Jankauskienė J., Kasiulevičiūtė- Bonakėrė A., Bliznikas Z., Novičkovas A., Breivė K., Žukauskas A., 2009. The effect of light emitting diodes lighting on cucumber transplants and after-effect on yield. Žemdirbystė– Agriculture, 96 (3), 102–118.

25- Brazaitytė A., Duchovskis P., Urbonavičiūtė A., Samuoliene G., Jankauskienė J., Sakalauskaitė J., Šabajevienė G., Sirtautas R., Novičkovas A., 2010. The effect of light-emitting diodes lighting on the growth of tomato transplants. Žemdirbystė–Agriculture, 97 (2), 89–98.

26- Juknys R., Duchovskis P., Sliesaravičius A., Šlepetys J., Januškaitienė I., Brazaitytė A., Ramaškevičienė A., Lazauskas S., Dėdeliene K., Sakalauskaitė J., Juozaitytė R., Kadžiulienė Ž., Dikšaitytė A., 2011. Response of different agricultural plants to elevated CO2 and air temperature. Žemdirbystė–Agriculture, 97 (2), 89–98.

27- Peirce, L.C. 1987. Vegetables characteristics, production and marketing. John Wiley and Sons. Inc. Toronto. Canada.

28- Opena, R.T. and M.L. Kyomo.(ed.) 1990. Vegetable Research and Development in SADCC Countries: Proceedings of workshop held at Arusha, Tanzania, 9 – 13 July 1990. AVRDC Publication No. 90 – 328.

29- Stevens, M.A. 1974. Varietal influence on nutritional value. P. 87 – 110. In P.L. White and N.SELVEY (eds.) Nutritional qualities of fresh fruits and vegetables. Futura Publ. Co., Mt. Kisco, N.Y.

30- Cobley, L.S. and W.M Steele. 1976. An Introduction to the Botany of Tropical Crops. Second edition. Longman Group Limited London.

31- Varela, A.M., A. Seif, and B. Lohr. 2003. A guide to IPM in tomato production in Eastern and Southern Africa. CTA/ICIPE/GTZ.

32- Naika, S., J.van Lidt de Jeude., M, de Goffau., M. Hilmi and B.van Dam.2005. Cultivation of tomato: production, processing and marketing. CTA. Series no 17.

33- Willcox JK, Catignani GL, Lazarus S. Tomatoes and cardiovascular health. Crit Rev Food Sci Nutr. 2003; 43(1):1-18.

34- Lazarus S.(2003), "Tomatoes and Cardiovascular health," Critical Reviews in Food Science and Nutrition, vol. 43, no. 1, pp. 1–18, 2003.

35- Y. Sharoni and Y. Levi, "Cancer prevention by dietary tomato lycopene and its molecular mechanisms," in Tomatoes, Lycopene & Human Health, A. V. Rao, Ed., pp. 111–125, Caledonian Science Press Ltd, Barcelona, Spain, 2006.

36- Vegetable Production Training Manual. 1992. Asian Vegetable Research and Develpoment Center. Taipei.Taiwan.

37- - Purseglove, R. 1988. Tropical Crops Dicotyledons. Fifth edition. Longman Publisher Singapore.

38- Bok I, M. Madisa, D. Machcha, M. Moamogwe and K. More (ed). 2006. Manual for vegetable production in Botswana. Department of Agricultural Research. Gaborone. Botswana.

39- Yoshida, S.; Forno, D.A.; Corck, J.H. and Gomez, K.A. (1976). Laboratory Manual for Physiology Studies of Rice Laguna.International Rice Res. Inst., Los Banos, and Manila, Philippines. pp. 61- 65.

40- El-Shahat, R.M. 1988. Prospects of Azolla as biofertilizers in Egyptian conditions. MSc. Thesis, Faculty of Agric., Ain Shams University, Cairo, Egypt, pp. 53-72.

41- Bindhu K.B., Effect of Azolla Extract on Growth Performance of Pisum Sativum. International Research Journal of Biological Sciences Vol. 2(10), 88-90, October (2013).

42- A.A. Glala, A.M. Hoda and Z.F. Fawzi. Improving Tomato Plant Growth, Health, Earliness, Productivity and Fruit Quality by Chemically Induced Systematic Resistance. Journal of Applied Sciences Research 1(5): 362-372, 2005.

43- Reitman S, Frankel S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957 Jul; 28(1):56-63.

44- Kulkarni K.R. (2006) Cholesterol profile measurement by VAP method. Clin Lab Med 26:787–802.

45- Lim J, Song KE, Song SH, Choi HJ, Koo SH, Kwon GC. Traceability Assessment and Performance Evaluation of Results for Measurement of Abbott Clinical Chemistry Assays on 4 Chemistry Analyzers. Arch Pathol Lab Med. 2016 May; 140(5):467-72.

46- Working Group on Lipoprotein Measurement (1995) NCEP recommendations for measurement of low-density lipoprotein cholesterol: executive summary. Clin Chem 41:1414–1426.

47- Henry, R. J., Cannon, D. C. and Winkelman, J. W. (1974).Clinical Chemistry Principles and Techniques , Harper and Row, New York 2nd.

48-Henry. R. J. (1974). Clinical Chemistry. Principles and Techniques. 2nd ed. Harper and Row, New York, p. 882.

49-Young, D. S. (1999). Effects of disease on clinical lab. Tests. Clinical Chemistry, 4th ed AACC 2001.

50- Steel, R. G. D., J. H. Torrie and D. A. Dickey (1997). Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw Hill Book Co., New York.

51- Bindhu K.B., Effect of Azolla Extract on Growth Performance of Pisum Sativum. International Research Journal of Biological Sciences Vol. 2(10), 88-90, October (2013).

52- A. S. Isah, E. B. Amans, E. C. Odion, and A. A. Yusuf. Growth Rate and Yield of Two Tomato Varieties (Lycopersicon esculentum Mill) under Green Manure and NPK Fertilizer Rate Samaru Northern Guinea SavannaInternational. Journal of Agronomy,Volume 2014, Article ID 932759, 8 pages.

53- Voutilainen S, Nurmi T, Mursu J, Rissanen TH. Carotenoids and cardiovascular health. Am J Clin Nutr. 2006 Jun; 83(6):1265-71.

54- Chen J, Song Y, Zhang L. Effect of lycopene supplementation on oxidative stress: an exploratory systematic review and meta-analysis of randomized controlled trials. J Med Food. 2013 May; 16(5):361-74.

55- Joanna Fiedor and Květoslava Burda. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. Nutrients. 2014 Feb; 6(2): 466–488.

56- Indira, D. ; Sarjan Rao, K. ; Suresh, J. ; Venugopal Naidu, K. ; Ravi, A., 2009. Azolla (Azolla pinnata) as feed supplement in buffalo calves on growth performance. Indian J. Anim. Nutr., 26 (4): 345-348.

57- Marwaha T.S., Singh B.V. and Goyal S.K., Effect of incorporation of Azolla on wheat (Triticum aestivum var HD-2329), Acta Bot. Indica, 20, 218-220 (1992).

58- Wagner G.M. Azolla, a review of its biological utilization, Bot. Rev., 63, 1-26 (1997).