International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Special Issue 1- ICMTBT | 2018 | 44-52
International Conference on Microbial Technology for Better Tomorrow ICMTBT
Held at D.Y. Patil College of Arts, Commerce and Science, Pimpri, Pune, 17-19 February
| Conference Proceedings | Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

ESTIMATION OF ANTIOXIDANT AND PHENOLIC CONTENT OF ORGANICALLY AND CONVENTIONALLY GROWN TOMATO CULTIVAR (Solan Lalima) UNDER MID-HILL CONDITIONS OF HIMACHAL PRADESH

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ABSTRACT

Indiscriminate use of chemical fertilizers and pesticides could cause adverse changes in biological balance as well as lead to an increase in incidence of cancer and other diseases, through the toxic residues present in the grains and other edible parts of plants. The need of the hour is to make tomato farming sustainable and profitable by gradually shifting from green to evergreen farming. Supporting a serious suitable option for small scale production system, organic agriculture holds another kind of promise in terms of overall productivity with ecological parameters. Keeping in view the above criteria, the present studies were carried out in the farmer's field (village Basal), 5 km away from Solan town, under mid hill conditions of Himachal Pradesh during the consecutive years (2013-2014), to study the effect of various organic and inorganic treatments on different antioxidant and phenolic content of tomato (cv. Solan Lalima), at the final harvesting stage. The results through HPLC confirmed that the combination of treatments were superior to the individual application of organic amendments as higher levels of phenolics (42.1%) and antioxidant activity (12.6%) was obtained as compared to the control (conventional system) (13.82% and 9.8%) respectively. In this research article antioxidant and phenolic activities of conventionally and organically grown tomatoes have been evaluated. It can be concluded from the present studies that by adopting appropriate combination of organic production technologies, nutritional values can be increased with better quality produce, improved soil health and nutrient status.

KEY WORDS

Antioxidant, Phenolics, Organic, Conventional, HPLC.

INTRODUCTION

Tomato (*Lycopersiconesculentum*) is one of the most important, popular and widely grown vegetable throughout the world. It belongs to family Solanaceae. It originated in Central and South America [1]. Tomato is playing an indispensable role in the economic upliftment of hilly area farmers in as an off-season crop in the mid-hills of Himachal Pradesh. Tomato produced in Himachal Pradesh during June to November becomes off-season vegetable in the markets of North Indian plains fetching very remunerative prices to farmers. High dry matter and soluble solids are desirable characteristic for the canned tomatoes industry since they improve the quality of the processed product [2]. Tomato being a heavy feeder crop demands constant supply of large amount of nutrients and water for its luxuriant growth. The global energy crises have increased the cost of chemical fertilizers and pesticides and this trend is expected to continue in near future also. It seems that the cost of chemical fertilizers will reach beyond the reach of marginal farmers. Tomatoes are important not only because of the large amount consumed, but also because of their high health and nutritional contribution to human being. Tomato

consumption has been shown to reduce the risks of cardiovascular disease and certain types of cancer, such as cancers of prostate, lung and stomach. The benefits of tomatoes and tomato products have been attributed mostly to the significant amount of lycopene contained, which constitutes 80 to 90 per cent of the total carotenoid content present in tomatoes [3]. The tomato fruits are rich source of vitamin C, the antioxidant properties of this fruit help in prevailing cancer and occurrence of heart diseases. The abundance of anthocyanin has made it more valuable vegetable crop. Increased interest in organic tomato production is imposed by the need to evaluate the quality and nutritional value of organic tomato [4]. Growing of crops on soil is the conventional practice in crop production, the search for an alternative means of media for cropping came as a result of increasing knowledge in plant nutrition as well as other serious difficulties observed in the use of soil in crop production. Soil possess numerous limitations for for plant growth due to presence of disease causing organisms (flora and fauna), poor drainage and aeration resulting from soil compaction and degradation due to soil erosion and leaching [5]. Soilless culture is the modern cultivation system of plants that use either inert organic or inorganic substrate through nutrient solution nourishment. Possibly it is the most intensive culture system utilizing all the resources efficiently for maximizing yield of crops and the most intense form of agricultural enterprises for commercial production of greenhouse vegetables [6, 7]. Several studies suggested soilless culture in the greenhouse as an alternative to traditional field production for high-value vegetable crops [8]. This protected cultivation system can control the growing environment through management of weather factors, amount and composition of nutrient solution and also the growing medium. Therefore, quality of horticultural crops grown through soilless culture improves significantly compared to conventional soil cultures [9, 10].

Tomato is one of the most important vegetable crops of Solan (HP) with 11 million tons of annual production [11]. At present, tomato production is mainly conventional [12] both in open field and in greenhouses in Solan (HP), which has diverse agro-climatic conditions (Fig 1.5) and due to favorable positioning in the Himalayan region, has a great scope for the promotion of organic farming. The state government formulated a policy on organic farming in 2010 and has covered 30,110 farmers with an area of 17,848 ha under organic farming with the future vision of converting 200 villages to complete bio-villages and 20,000 vermi-compost units with 50 per cent assistance will be given. However, government has already initiated the organic cultivation, registration and certification process to use organic fertilizers beside the inorganic fertilizers in tomato production, but the farmers are still not aware about the incorporation of organic recommendations.

MATERIALS AND METHODS

2.1 Experimental location

The experimental trial was set up in the farmer's field, located at village Basal, 5Km away from Solan town, under Solan block of Solan district, Himachal Pradesh at an elevation of 1270m above mean sea level located 30-52' North and latitude 77-11' east.

2.2 Weather data of experimental site

The experimental area lies under the sub-temperate, sub- humid mid-hill agro-climatic zone of Himachal Pradesh, where summers are moderately hot during May-June, while winters are severe during December-January. The average rainfall in this area ranges from100-300cm, most of which was received during monsoon months of July and August.

2.3 Experimental layout

The experiment trial consisted of a primary nursery stage and a secondary field trial. The treatments and procedures followed are separately discussed under nursery and field experiment sections. The six organic treatment were compared with the control during entire course of study.

2.4 Sources of organic amendments and inputs

2.4.1 Manures and Bio-fertilizers used

2.4.1.1 Manures used:

- FYM (Farm yard manure) and VC (Vermicompost) were procured from the farmer's field having compost pits and vermi- bed.

2.4.1.2 Biofertilizers and Biocontrol agents:

- AZO (*Azotobacter*), PSB (phosphate soluabilizing bacteria), Neem cakes, *Trichodermaviridae, Pseudomonas fluorescens*and Asafetida were procured from Poabs Green Pvt. Limted- Kerela.

2.4.1.3 On-farm inputs:

- Bijamrit and Jeevamrit were prepared freshly on the farm.

2.5 Chemicals used

All the chemicals used were of analytic grade and were purchased from Sigma Aldrich.

Nursery parameters

2.6.1Organic package of practices adopted for growing healthy tomato nursery.

The Seeds of tomato (cv. *Solan lalima*) were sown in plastic trays (13x9=117 seeds). The nursery was set up with three replication and six designed organic treatments. The control was laid according to farmer's practice in field in a seed bed (1m x 3m). The different combination of media were used which contained both soilless and soil growth media mixed with various organic manures and bio-fertilizers. The detailed description of various treatments is given in Table 1.

2.6.1.1 Seed source, seed variety and seed rate used

2.6.1.1.1Seed source:

Procured from Department of Vegetable crops Dr. Y.S. Parmar -UHF Solan.

2.6.1.1.2 Tomato variety used:

Solan lalima (open pollinated).*Solan lalima* is an open pollinated and indeterminate variety of tomato having superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being an open pollinated variety, it is a suitable option for organic cultivation.

2.6.1.1.3 Seed rate: 400 g/ha (40 gm/bigha)

Table 1: Detailed description of various treatments followed during tomato nursery raising

S.No.	Treatments	Combinations of various growth media combined with organic practices
1	**T_1**	FYM + Soil (1:1)
2	**T_2**	FYM+ VC+ Soil (1:1:1)
3	**T_3**	FYM + coco peat + VC + Vermiculite + *Azotobater*(1:1:1:1:1)
4	**T_4**	FYM + coco peat + Vermiculite + Azotobacter (1:1:1:1)
5	**T_5**	FYM + soil + *Azotobacter* (1:1:1)
6	**T_6**	FYM + *Azotobacter*(1:1)
	T_7	FYM + soil + no seed treatment + Drenching with Mancozeb and
7	**Control (Farmer's practice)**	Carbendazim. (2.5g/L and 0.5g/ L of H_2O)

FYM: Farm Yard manure; VC: Vermicompost

2.6.2 Seed treatment

The seeds were treated with Beejamrut (6g/40g seed) and *Trichodermaviridi*(0.32g). The seeds weredried in the shade and again treated with a mixture of *Azotobacter* and PSB (0.8g each). Finally dried the seeds in shade and sown within 8 hrs of treatment.

2.6.2.1 Treatment of trays used for raising nursery

The trays were treated with 1:7 Formalin

2.6.2.2 Seedling treatment

- Neem spray (7g/l) was given once for 15 days old seedlings to protect seedlings from sucking pests like white fly and thrips.
- Drenching was done with *Pseudomonas fluorescence* (10g/l) before transplanting to prevent foliar diseases.
- Dipping of root portion of seedling in Asafoetida suspension (100g in 5l of water for 20 min) was done to prevent soil-borne pathogens causing wilt diseases before transplanting. Twenty-five days old tomato seedlings were transplanted to the main experimental field.

RESULTS

Observations were recorded on the following aspects:

- Seedling germination %=No. of seedlings emerging out of total germinated.
- Root length (cm)
- Shoot length (cm)
- Number of roots
- Seedling vigor = Seedling germination (%) x (root length (cm)+ shoot length (cm)
- Incidence of pre and post-emergence damping-off was calculated as:

Pre-emergence rot (%):

Total number of seeds sown-germinated seeds

Post emergence rot (%):

percentage of toppled plant out of germinated ones

2.8 Field parameters

2.8.1Experimental design of the field

The experiment was laid out in a RBD (Randomized Block Design) with eight treatments replicated five times. The design consisted of 40 plots (1m x 3m) in which tomato seedlings were planted at a distance 90 cm x 30 cm having 24 plants per plot. The six (T_1-T_6)

organic treatments were applied in different consolidated blocks separated at a distance of 7m from the control. The doses of the manures and biofertilizers have been formulated by carrying out the soil and manure analysis and doses recommendations prescribed in organic package of tomato crop.

T_1 --- FYM @ 312q/ha +*Tricho dermaviride* @ 4kg/ha

T_2---VC @ 78q/ha +*Tricho dermaviride* @ 4kg/ha

T_3----VC @ 78q/ha + *Azotobacter*+ PSB + *Tricho dermaviride* (4kg/ha each)

T_4----FYM @312q/ha + *Azotobacter* + PSB + *Tricho dermaviride*@ (4kg/ha)

T_5---- PSB+ *Tricho dermaviride* (4kg/ha)

T_6-----*Azotobacter* + *Tricho dermaviride* (4kg/ha)

T_7 ---- (conventional practices) FYM@ 250q/ha + chemical fertilizers (CAN + Urea + pesticides (50-60 No. of sprays) (Farmer's practice).

2.9 Soil analysis

Before commencement of the experiment, the soil of the experimental area and manures used were analyzed for physiochemical properties with following method. The soil analysis revealed low status of available Organic Carbon, Nitrogen and Potassium and high percentage of Phosphorus. To combat with low and high percentage of NPK and organic carbon, 25 percent high and low application of manures and biofertilizers were used in accordance with the recommended packages.

2.10 Field Operation Protocol followed

2.10.1 Random selection from the field experiment

A random selection of ten plants was considered from each bed. On a whole 200 plants were considered under field parameter analysis.

2.10.2 Determination of Total Antioxidant activity

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of foodstuffs. The total antioxidant activity was determined by DPPH (1-1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging Assay as described by [13]. The molecule of 1,1-diphenyl-2-picryl-hydrazyl was characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also give rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520nm.

2.10.2.1 Sample preparation

0.5g ground macerated tomato powder both for organic and conventional (crushed with liquid nitrogen) was weighed and extracted with 50 ml 80% aqueous methanol on an ultrasonic bath for 20 minutes.The solubility of dehydrated samples was checked in distilled water, methanol and distilled water-methanol solution. The samples were most soluble in methanol, so these were used for DPPH assay.

2.10.2.2Standard preparation

Ascorbic acid (1mg/ml) was used as standard and it was prepared in three different solvents (80% methanol; 100% Methanol and water) to work out the lowest IC50 (inhibitory concentration) value and high antioxidant activity.

DPPH stock solution (0.1mM) was prepared by dissolving 39.4 mg in 1000ml of methanol. Stored in dark coloured bottle.

Procedure followed

Three ml of 0.1mM DPPH solution was added to 1ml various sample concentration (100-1000microgram/ml) of extract and ascorbic acid working standard (10-100microgram/ml). Absorbance was recorded after 30minutes at 517nm. Finally, the per cent inhibition activity was calculated from the following formula:

% inhibition activity = 100 x (A c – A s)/ A c

(Where Ac is absorbance of control; As is absorbance of sample or standard)

Finally, IC50 (Inhibitory concentration: substrate concentration to produce 50% reduction of the DPPH) values were calculated. The organic and control samples with low IC 50 values (high antioxidant activity) were further carried out for HPLC analysis (Table A 3.4).

2.10.3 Determination of Total phenolics

2.10.3.1Sample preparation

A sample measuring 0.5g ground macerated tomato powder (crushed with liquid nitrogen) was weighed and extracted with 50 ml 80 per cent aqueous methanol on an ultrasonic bath for 20 minutes.

Procedure followed

The content of Total Phenolics was determined using the Folinciocalteauassay [14] with Gallic acid as a calibration standard. The FC assay was carried out by pipetting 100 ml of tomato extract into a 12ml amber vial. This was followed by addition of 7.9ml of water. This mixture was vortexed for 10–20 seconds and 500 ml of FC reagent was added to it. The mixture was then

vortexed for an additional 20–30 second and 1.5ml of filtered 20 per cent sodium carbonate solution was added after 1 min and before 8 min of addition of the FC reagent. This was recorded as time zero. The mixture was then vortexed for 20–30 seconds after addition of sodium carbonate. The samples were subjected to HPLC analysis (Table A 3.5).

2.10.4 Total antioxidant activity and total phenolics

One parameter that has been introduced recently for the interpretation of the results from the DPPH method is the "efficient concentration" or EC50 value (otherwise called the IC_{50} value). Table 1 indicated various significant effects of organic amendments and biofertilizers on the antioxidant activity. The pooled data summarized in Table 2, showed a tremendous difference in IC50 values during both the years of study (2013 and 2014). Treatment T_7 was recorded with highest (6891.33microgram/ml) IC_{50} value, whereas, minimum IC_{50} value (74.55 microgram/ml) was recorded under treatment T_3. The results of both the experimental years revealed that T_3 was recorded with lowest IC_{50} values (high antioxidant activity), viz. 94.6 microgram/ml during 2013 and 54.5 microgram/ml during 2014, respectively.

Table 2: **Effect of seed priming with different combinations of organic treatments coupled with BCA's on total antioxidant (%) of tomato cv. Solan Lalima (2013-14).**

Treatments	Treatments Description	DPPH (IC50)(microgram/ml)		
		2013	**2014**	**Mean$\pm SD$**
T_1	FYM @ 312q/ha +*Trichodermaviride*@ 4kg/ha	363.13±5.42	256.31±0.22	309.72±2.82
T_2	VC @ 78q/ha +*Trichodermaviride*@ 4kg/ha	421.43±0.40	368.30±0.51	394.86 ±0.45
T_3	VC @ 78q/ha + *Azotobacter*+ PSB + *Trichodermaviride*(4kg/ha each)	94.6 ±0.51	54.5±0.43	74.55 ±0.47
T_4	FYM @ 312q/ha + *Azotobacter* + PSB + *Trichodermaviride*@ (4kg/ha)	357.06 ± 0.11	241.13 ±0.11	299.09 ±0.11
T_5	PSB+ *Trichodermaviride* (4kg/ha)	421.4 ±0.05	121.48±0.09	271.44 ± 0.07
T_6	*Azotobacter* + *Trichodermaviride* (4kg/ha)	3651.06 ± 0.11	3259.2 ±0.30	3455.13 ±0.20
T_7	**(**conventional practices)	1279.56±0.23	1023.1±0.17	6891.33 ±0.20
T_8	Recommended POP	2437.13 ±0.11	1387.1 ±0.03	1912.15 ± 0.07
	df	**24**		
	SE(M)	3.747	0.082	
	SE (d)	1.58	0.233	
	CD value (0.05)	4.41	0.651	

SD: standard deviation; Level of significance (at $\propto = 0.05$) by Fisher (F) test; SE(M): Standard error mean; SE(d): Deviation from SD: standard deviation; CD: Critical difference; df: degree of freedom

However, the highest IC_{50} values were observed under control (1279.56 microgram / ml and 1023.1 microgram / ml during 2013 and 2014). The data pertaining to first year of study (2013) showed considerable increase in IC50 values in comparison to the second year, thus indicating a rise in antioxidant potential over the preceding year. However, the organic treatments were recorded with maximum antioxidant potential over the control practices.

2.10.5 HPLC analysis for total antioxidant activity

The HPLC analysis (Table 3) was carried out to quantify total ascorbic acid (Fig 1a) of organic treatments in comparison to the control treatment (Retention time-10.51 min). It revealed that the organic sample (Fig 1b) was recorded with 12.6 per cent purity as compared to control (T7) (Fig 1c) with 9.8 per cent purity. The analysis clearly indicated that the organic treatments were higher in terms of antioxidant content in comparison to the control.

Figure 1(a): HPLC Chromatogram (Standard) of Ascorbic acid (RT 10.51 min).

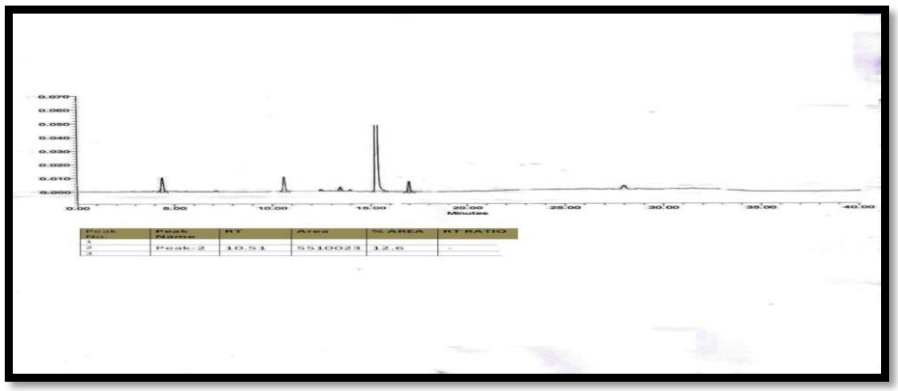

Figure 1 (b) HPLC chromatogram of puified organic tomato extract showig peak (peak 2) ofascorbic acid

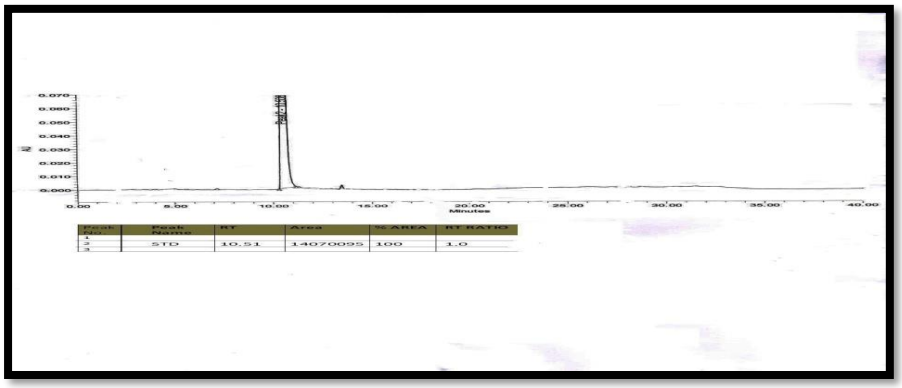

Figure 2: HPLC Chromatogram (Standard) of Gallic acid (RT 28.3 min).

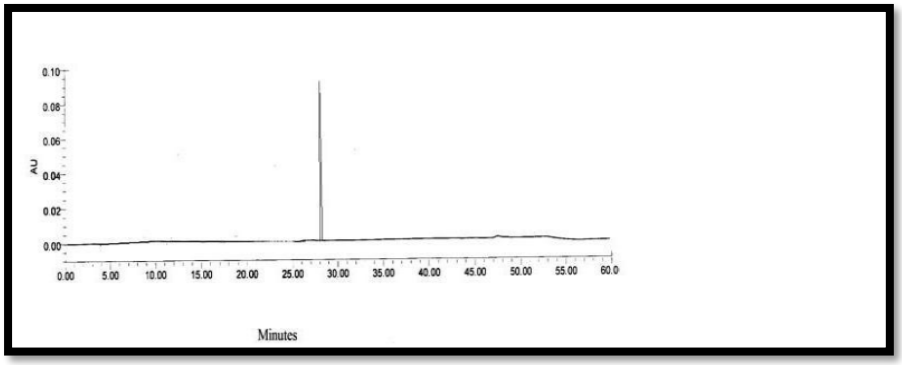

Figure 2 HPLC chromatogram of purified conventional tomato extract showing peak (peak 4) of ascobic acid

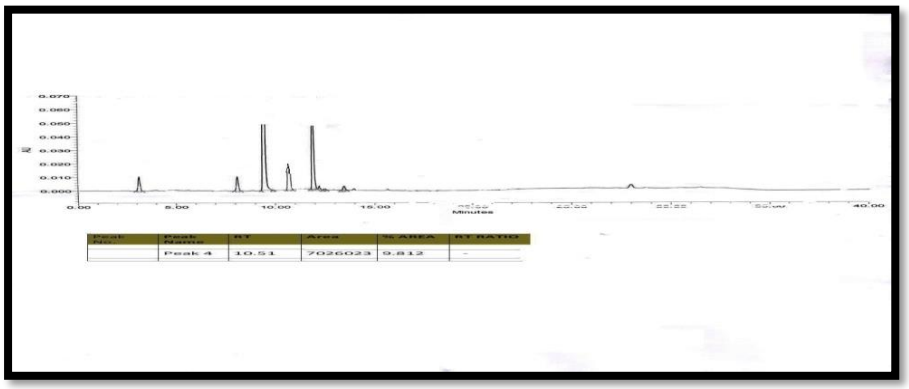

Table 3: HPLC method development for analysis of total antioxidant activity.

S.No.	Analyte	Retention time	% Area
1.	Std. Ascorbic acid	10.51	100%
2.	Organic sample (T3)	10.51	12.6%
3.	Farmer's practice (C)	10.51	9.8%

4.3.5 HPLC analysis for total phenolics

The HPLC analysis (Table 4) carried out to quantify total ascorbic acid (Fig 2). The best organic sample and control sample when subjected to HPLC analysis (Table 3) revealed highest total phenolics corresponding to 42.1 per cent. However, the control was recorded with 13.82 per cent (Fig 2 c), which was at lower hand in comparison to the organic treatment.

Table 4: HPLC method development for analysis of total phenolics.

S.No.	Analyte	Retention time	% Area
1.	Std. Gallic acid	28.3	100%
2.	Organic sample (T3)	28.3	42.1%
3.	Farmer's practice (C)	28.3	13.82%

DISCUSSION

Vermi-compost application in combination with biofertilizers and biocontrol agent to tomato crop registered significantly higher total antioxidant activity and phenols as compared to the conventionally grown. HPLC analyses of organic sample in the present study reported an increase of 2.8 per cent in total antioxidant activity over control, however a remarkable increase in total phenolics was recorded which was about 28.2 per cent over the control. Similar increase in antioxidant activity in organically cultivated crops was reported by [15] which may be due to the reason that organic production methods which are limited in the use of insecticides, herbicides and fungicides compared to conventionally cultivated plants may need to synthesize their own chemical defense mechanisms and the increase in antioxidant activity has been attributed to this need. The inoculation with bacterial mixtures provided a more balanced nutrition for the plants and the improvement in nitrogen and phosphorus uptake via roots was the major mechanism of interaction between plants and bacteria. Because many of these compounds are produced by plants to protect themselves against attack and damage by disease. If a plant is subjected to higher levels of stress, it will accumulate higher levels of secondary compounds. Thus, if exogenous chemicals such as insecticides and fungicides are reduced or avoided, a greater reliance on the plants' own natural protection systems will result in the accumulation of higher levels of these plant defense related (secondary nutrient) compounds [16]. Organic farming is generally characterized by a lower level of nitrogen supply compared to non-organic [17]. This can lead to an earlier completion of vegetative growth in organic plants and an earlier onset of maturity processes than in non-organically produced plant products [18], as phytonutrient metabolites are often synthesised primarily during the maturation of plant products, this may result in higher concentrations of these compounds in organically grown foods.

CONCLUSION

The research work conducted during two consecutive years (2013-014) illustrates the complexity of soil and the importance of good soil management techniques in agricultural practices. Major themes include increasing and protecting soil fertility, protecting soil from erosion by improved drainage system, the importance of soil biology and soil organic matter, and how organic fertilization coupled with biofertilizers can contribute to food quality and productivity. The organic farming methods used to protect soil fertility and minimize soil erosion include barrier crops, mulching, green manuring, application of Farm Yard Manure and Vermi-compost, use of botanicals for Insect Pest and Disease management, on farm generated inputs and conservation tillage.

Organic farming relies on a high level of biological activity in the soil and at the same time contributes to increase the diversity and abundance of those same soil microorganisms. Some of the benefits of this includes: improved uptake of minerals and enhancement of the nutrient supply, improved crop vigour, reduced nutrient leaching, improved soil structure and water infiltration,

and increased resistance to soil-borne pest and diseases.

Soil communities rely on Soil Organic Matter (SOM), while the nutrients in SOM are made available to plants by those organisms that rely on it for food. Increased level of soil organic matter (SOM) is also a characteristic of good soil as it serves several important functions that are especially important to organic agricultural systems. These functions include improved soil structure, aggregation, water infiltration and over-all productivity. Furthermore, organic agriculture soil management often increases SOM, over time, whereas conventional agriculture generally lowers it. Therefore, increasing SOM seems to be an important goal for organic farmers. Finally, as the outcome of the studies indicates that the content of vitamins, antioxidants, phenolics and bioactive compounds were higher under organically grown tomato. Similarly, the shelf life of organically grown tomatoes was higher than the conventional ones. Also, the pesticide residues were minimum under organic treatments as compared to the conventionally grown tomato, which is one of the most important outcomes of the studies.

At the end this can be concluded that, the organic system of cultivation is good for environment, human health having attractive returns to the farmers.

CONFLICT OF INTEREST

Conflict of interest declared none.

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