Impact of *Kunapajala* treatment from *Vrikshyaurveda* on leaves of tomato relative to conventional and organic farming techniques.

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ABSTRACT

Kunapajala is a fermentation product of easily available ingredient and it works as a natural plant growth booster. Experiments were conducted with tomatoes as using pot culture method for NPK farming, organic farming and *kunapajala* treatment in organic way. The results obtained under the *kunapajala* treatment were more effective for inducing number of leaves per plant and biomass of leaves compared to conventional farming and organic farming. Leaf area was same under both conventional farming and *kunapajala* treatment. Leaves showed highest relative water content (RWC), osmotic potential (OP) of cell sap, total chlorophylls, chlorophyll stability index, carotenoids and xanthophylls and lowest percentage of membrane injury under *kunapajala* treatment followed by NPK farming and organic farming. Bio-organic study showed that *kunapajala* had upper hand, followed by organic farming and conventional farming in terms of soluble proteins, total carbohydrates, polyphenol, proline, glycine betain and ascorbic acid. The antioxidant property of tomato leaf was highest with *kunapajala* treatment compared to NPK farming and organic farming as revealed by activity of enzymes viz. catalase, peroxidase, polyphenol oxidase, IAA oxidase and super oxide dismutase. This overall picture shows that *kunapajala* treatment is superior to NPK farming and organic farming as it brings about physiological, biochemical and enzymatic enhancement in the leaves of tomato under organic farming conditions.
Keywords: Bio-organics, *kunapajala*, leaf, oxidative enzymes, organic farming, tomato.

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INTRODUCTION

Vegetables are important in human diet for their vitamin and mineral contents which are essential for metabolic processes taking place within the human body. Tomato is a solanaceous fruit vegetable which is available round the year. It is cultivated in the tropics and subtropics. It is believed that consumption of one tomato per day enhances the health status of individuals and it is considered to be important in diets as it is quite high in nutritive value (Jagadeesha, 2008). Tomato is the top source of Vitamin A and C. It also contains a significant amount of dietary fibers, beta-carotene, iron, lycopene, magnesium, niacin, potassium, phosphorus, riboflavin and thiamine.

India has made spectacular breakthrough in production and consumption of fertilizers during the last four decades. But consumption of chemical fertilizers will be quite a limiting factor for increasing agricultural production in future. The costs of fertilizers has been increasing astronomically to such an extent that it is getting out of reach of the small and marginal farmers. It has become impractical to apply such costly inputs for a crop of marginal returns. Moreover, the imbalanced and continuous use of chemical fertilizers is leading to reduction in crop yields and adverse effect on soil health. Therefore, there is an urgent need to reduce the use of chemical fertilizers and at the same time increase soil fertility which are needed to enhance crop yield and quality levels.

Application of organic manures has been a noble and traditional practice of maintaining soil health and fertility. The importance of organic manures is realized because of their inherent capacity to supply most essential nutrients for a balanced nutrition to the crop. Organic nutrients generally facilitate crop rooting, improve water retention capacity and result in an even distribution of nutrients in soil profile. Organic farming is meant for sustainable agriculture. It is a unique production management system which promotes the health of soil leading to production of healthy crops with better nutrient quality. Organic food (a.k.a Green food) catches 2 to 5 times more market price (Subramanian, 2006). Unfortunately the productivity of organically grown crop is relatively less.

Liquid biofertilizers play a vital role in organic farming leading to green food production which is safer, healthier and tastier. The concept of biofertilizer is mentioned in *Vrikshyaurveda* under the generic name '*kunapajala'* by Surpala (1000 AD) in eastern India as cited by (Sadhale, 1996). Firminger (1864) mentioned the beneficial use of liquid manure *kunapajala* for vegetable

cultivation. According to Neff *et al*. (2003), the reason behind the effectiveness of *kunapajala* is that the ingredients of *kunapajala* have been fermented, which means the proteins, fats, carbohydrates etc. are broken into simple low molecular weight products. Therefore, nutrients from *kunapajala* become available to the plants faster than from the traditionally applied organic matter. In addition, Patil (2007) mentioned that there is always a danger of passing on dormant pathogen to fields with plant based compost. But this is avoided by *kunapajala* because the *kunapajala* ingredients are cooked and fermented. So, it is concluded that the use of *kunapajala* enhances vegetative growth which leads to better yield with increased disease resistance under organic farming condition (Deshmukh et al. 2011). Nene (2006) mentioned that, there is no fixed proportion for the ingredients of *kunapajala* and further research is needed to standardize the procedure and test it on crops. Mishra (2007) pointed out that *kunapajala* can be a good substitute to synthetic fertilizers. So, there is need to standardize *kunapajala* formulations and time and frequency for *kunapajala* application Shukla and Naik (1993) mentioned that the adequate supply of nutrients can increase the yield, fruit quality, fruit size, keeping quality, colour and taste of tomato. So, in order to improve the quality as well as quantity of tomato, the technology should be developed which eventually fulfills the need of both growers and consumers in organic ways.

To address this shortfall, it is intended to study the impact of *kunapajala*, a liquid biofertilizer from *Vrikshayurveda* on leaf of tomato (*Lycopersicon esculentum* L. cv. Selection 22) grown organically and to compare it with NPK farming and organic farming.

MATERIALS AND METHODS

The experiments were conducted at P.G. Research Centre, Department of Botany, Tuljaram Chaturachand College, Baramati, Dist. Pune, (M.S.) India (between 18º3’ N to 18º12’ latitude, 74º13’ E to74º40’ E longitude and 548 m above mean sea level), in shade house using pot culture method. Earthen pots (40×40 cm) were used for the experimentation. NPK farming (T-1) was carried out by giving the treatment of NPK dose. Soil and vermicompost in 9:1 ratio were used for organic farming (T-2). *Kunapajala* (T-3) was prepared as per formula of Deshmukh *et al.*, (2011) and treatment was given to plants (20 DAS) for five times at the interval of 10 days by soil application method. The pots without any treatment were considered as control (T-4). The seeds of tomato (*Lycopersicon esculentum* L. cv. Selection 22) were sown randomly in these pots. The experiment was conducted in 20 replications.

Morphology of leaf of tomato was studied using routine laboratory methods. Freshly harvested third and fourth leaf from top of ten different plants were collected, cleaned properly and blotted dry. These were cut to small pieces and composite sample was prepared. This composite sample of leaf was used for physiological analysis. Osmotic potential of cell sap (OP) and membrane stability were measured by the methods proposed by Janardhan *et al*. (1976) and Premchandra *et al*. (1990) respectively. The photosynthetic pigments like chlorophylls and carotenoids were estimated by methods proposed by Arnon (1949) and Jensen (1978) respectively. The biochemical constituents were analyzed using the methods proposed by Lowry *et al*. (1951) for soluble proteins, Sadasivam and Manikam (2005) for total carbohydrates and for ascorbic acid, Grieve and Grattan (1983) for glycine betaine and Bates *et al*. (1973) for proline. The DNA and RNA were estimated according to the method of Ashwell (1957). The enzyme catalase was assayed according to the method described by Luck (1974). The activity of peroxidase enzyme was determined according to the method of Malik and Singh (1980) and that of polyphynol oxidase by Mahadevan and Shridhar (1982). Activities of super oxide dismutase and IAA oxidase were analyzed using the methods proposed by Giannopolitis and Ries (1977) and Tang and Bonner (1947) respectively.

RESULTS AND DISCUSSION

Impact of conventional farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on morphological parameter and water relations in leaf of tomato as at 60 DAS is shown in Table 1. The comparative study of different treatments with control showed that there was significant increase in number of leaves per plant, leaf area and leaf area index with T-1 (44 %, 121 % and 40 %) , with T-2 (25 %, 81 % and 50 %) and with T-3 (46 %, 121 % and 140 %) respectively. Photoplate 1 shows the impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* (T-3) treatment on morphology in leaves of tomato (*Lycopersicon esculentum* L. cv. Selection 22) as at 60 DAS. Plant size and leaf area are important variables in breeding for crop adaptation to water-limited environments. Singh *et al*. (2008) pointed out that leaf area index had significant positive correlation with total dry matter, total chlorophyll content, seed yield and harvest index. So, increase in leaf area index under T-3 treatment is significant for the productivity of tomato plant. As compared to the control, the total biomass was increased by 67% in T-1, 78 % in T-2 and 141 % in T-3 respectively on fresh weight basis and 97 %, 114 % and 290 % in T-1, T-2 and T-3 respectively on dry weight basis. Kumar *et al.* (2001) and Singh

et al. (2004) remarked that the attainment of biomass was significantly and positively correlated with seed yield. T-3 was effective in enhancing the morphological parameters of the leaves of tomato plant followed by T-1 and T-2. Relative water content (RWC) and osmotic potential (OP) of cell sap showed maximum increase under the influence of T-3 (30 % and 26 % respectively), as against T-1 (8 % and 26 % respectively) and T-2 (12 % and 6 % respectively) compared to the control. About 95 % of water absorbed by plant is lost through transpiration and about 5 % of absorbed water is available for plant metabolism. So increase in RWC under present investigation is very significant for plant metabolism. Moreover, Sinclair and Ludlow (1985) reported that, plant metabolism is dependent on leaf water status. RWC has been proposed as a selection criterion for drought tolerance in many crops as reported by Schonfeld *et al.* (1988) in barley and Martin *et al* .(1989) in wheat. In the present study values of RWC and OP were highest in T-3 followed by T-1 and T-2 which played a significant role in decreasing membrane injury in T-1 by 17.1 %, in T-2 by 16.88 % in T-3 by 15.94 % compared to the control.

Table 2 reports the impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on photosynthetic pigments in leaves of tomato as at 60 DAS. A leaf is specialized for the process of photosynthesis. Productivity of crop plants is intimately associated with the photosynthetic pigments. Chlorophyll content is a good index to meet an overall evaluation of any crop for its photosynthetic ability. So, the productivity of any crop is linked with chlorophyll content, which decides the solar energy harnessing ability of plant. As compared to the control, chlorophyll ‘a’ showed nearly the same increase in T-1 and T-3 by 49 % followed by T-2 (29 %). However, chlorophyll ‘b’ showed greater increase in T-3 (23 %) followed by T-1 (22 %) and T-2 (10 %) respectively. Chlorophyll ‘b’ absorbs energy from light and transfers it to chlorophyll ‘a’. The total chlorophylls and chlorophyll stability index increased by 37 % and 53 % in T-1, 21 % and 62 % in T-2 and 38 % and 67 % in T-3 treatment. The chlorophyll stability index (CSI) is an important index for screening plant tolerance to abiotic stresses (Gomaz NL and Rangasamy, 2002 and Yagameena, 2004). Carotenoids and xanthophylls increased by 5 % and 9 % respectively in T-1, 2 % and 52 % respectively in T-2 and 6 % and 68 % in T-3 compared to the control. Carotenoids react directly with singlet oxygen to detoxify it or they can quench the chlorophyll sensitizer and thus prevent singlet oxygen production (Foote, 1976). They react with singlet oxygen to produce carotenoid triplet which then decays, harmlessly producing heat rather than any toxic product.

Table 1:Impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* (T-3) treatment on morphology and water relations in leaves of tomato (*Lycopersicon esculentum* cv. Selection 22) as on 60 DAS.

Sr. No.	Parameters	Control	Treatments		
			NPK farming (T - 1)	Organic farming (T - 2)	*Kunapajala* (T - 3)
01	Number of leaves / plant	8.23^{c} ±0.66	$11.84^{a,b}$ ±0.12	10.31^{b} ±0.11	12.01^{a} ±0.33
02	Leaf area (cm^2)	162.3^{c} ±1.2	358.5^{a} ±1.54	294.1^{b} ±1.64	358.5^{a} ±1.44
03	Leaf area index	0.10^{c} ±0.5	0.14^{b} ±0.5	0.15^{b} ±0.2	0.24^{a} ±0.5
04	Biomass :				
	A. Fresh wt. (g)	3.826^{d} ±1.88	6.396^{c} ±1.45	6.820^{b} ±1.88	9.217^{a} ±1.74
	B. Dry wt. (g)	0.621^{d} ±0.86	1.224^{c} ±0.64	1.328^{b} ±0.35	2.424^{a} ±0.77
05	Relative water content (%)	47.18^{d} ±0.52	51.08^{c} ±0.47	52.76^{b} ±0.32	53.17^{a} ±0.31
06	Osmotic potential of cell sap (- bar)	-4.816^{d} ±0.83	-3.553^{a} ±0.34	-4.537^{c} ±0.48	-3.550^{b} ±0.24
07	Membrane injury (%)	25.0^{a} ±0.79	17.1^{b} ±0.36	16.88^{c} ±0.13	15.94^{d} ±0.52

Data presented in the table are mean ± SE scored after 60 days from 10 plants per treatment and experiment repeated thrice. Mean followed by same letters are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.

Table 2: Impact of NPK farming (T_1), organic farming (T_2) and *kunapajala* treatment (T_3) on Photosynthetic pigments in leaves of tomato (*Lycopersicon esculentum* cv. Selection 22) as on 60 DAS.

Sr. No.	Parameters	Control	Treatments		
			NPK farming (T - 1)	Organic farming (T - 2)	*Kunapajala* (T - 3)
01	Chlorophyll a (mg / g fresh wt.)	79.55^{c} ±1.03	118.78^{a} ±1.47	102.35^{b} ±1.25	118.73^{a} ±1.11
02	Chlorophyll b (mg / g fresh wt.)	59.55^{c} ±0.90	$72.78^{a,b}$ ±1.154	65.49^{b} ±1.42	73.23^{a} ±1.12
03	Total chlorophylls (mg / g fresh wt.)	139.1^{c} ±1.34	191.56^{a} ±1.78	167.84^{b} ±1.25	191.96^{a} ±1.13
04	Chlorophyll stability Index	0.57	0.87	0.92	0.95
05	Carotenoids (mg/ 100g)	19.91^{c} ±0.33	$21.01^{a,b}$ ±0.56	20.37^{b} ±0.35	21.14^{a} ±0.47
06	Xanthophylls (mg/ 100g)	4.32^{d} ±0.29	4.72^{c} ±0.63	6.56^{b} ±0.13	7.28^{a} ±0.47

Data presented in the table are mean ± SE scored after 60 days from 10 plants per treatment and experiment repeated thrice. Mean followed by same letters are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.

Table 3: Impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on bio-organics in leaves of tomato (*Lycopersicon esculentum* cv. Selection 22) as on 60 DAS.

Sr. No.	Parameters	Control	Treatments		
			Conventional farming (T - 1)	Organic farming (T - 2)	*Kunapajala* (T - 3)
01	Soluble proteins (g 100 $^{-1}$ g fresh wt.)	7.60^{c} ±0.66	12.7^{b} ±0.11	12.7^{b} ±0.33	13.2^{a} ±0.1
02	Total carbohydrates (g 100 $^{-1}$ g fresh wt.)	12.1^{d} ±0.22	19.0^{a} ±0.5	14.44^{c} ±0.22	18.80^{b} ±0.5
03	Polyphenols (g 100 $^{-1}$ g fresh wt.)	3.008^{d} ±1.6	6.883^{b} ±1.20	6.412^{c} ±1.33	8.930^{a} ±1.33
04	Proline (g/ 100 g dry wt.)	0.13^{d} ±0.32	0.16^{c} ±0.64	0.19^{b} ±0.83	0.22^{a} ±0.5
05	Glycine betaine (g/100 g dry wt.)	0.214^{c} ±0.66	$0.80^{a,b}$ ±0.36	0.60^{b} ±0.51	1.10^{a} ±0.16
06	Ascorbic acid (mg/ 100g fresh wt.)	11.36^{d} ±1.88	15.10^{b} ±1.65	13.77^{c} ±1.35	15.60^{a} ±1.69

Data presented in the table are mean ± SE scored after 60 days from 10 plants per treatment and experiment repeated thrice. Mean followed by same letters are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.

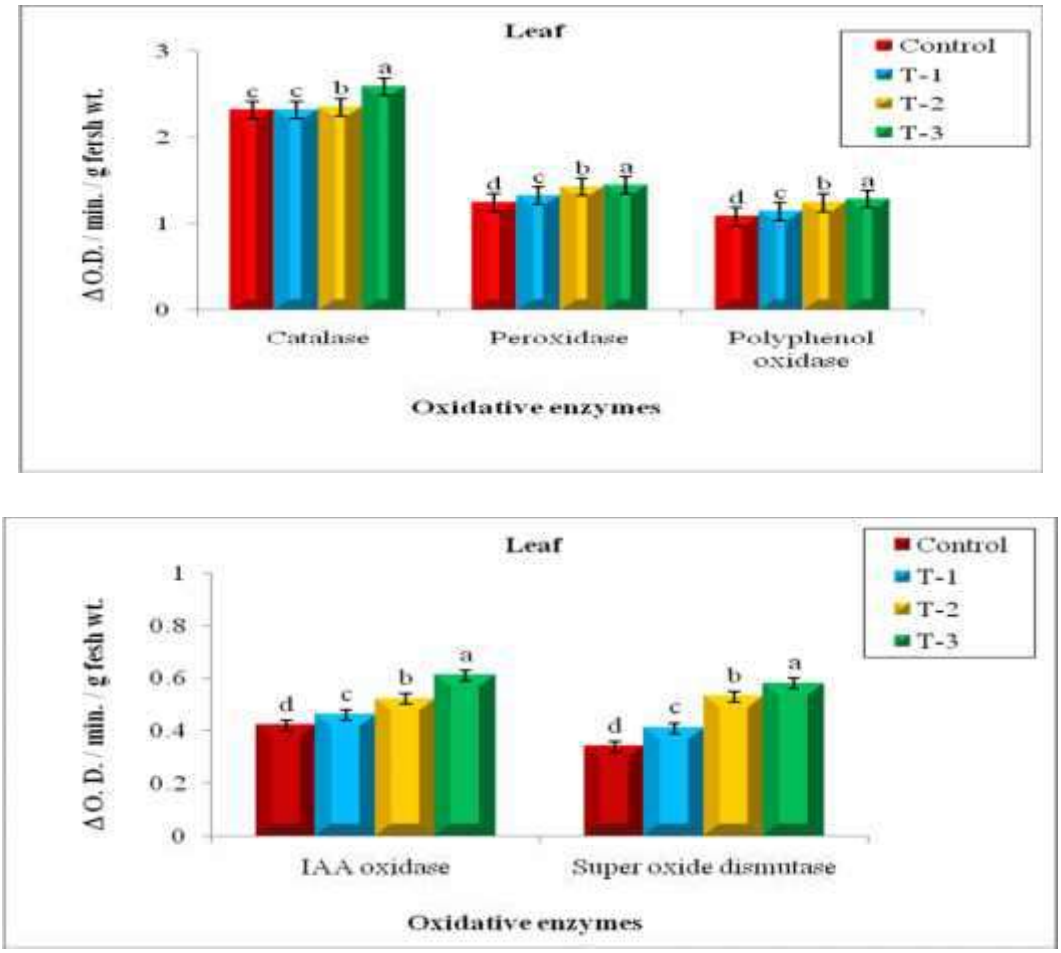
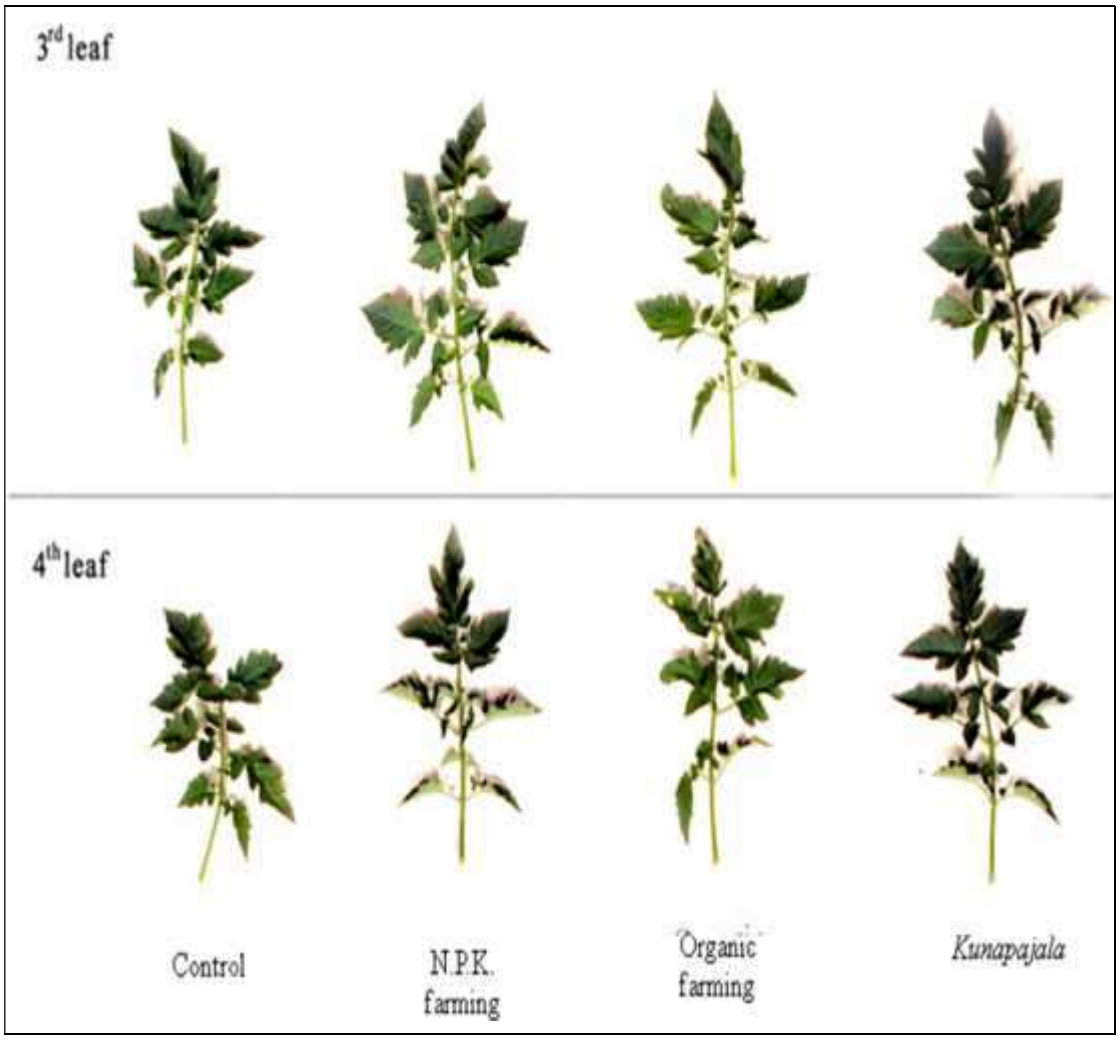


Fig. 1: Impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* (T-3) on enzyme activity in leaves of tomato (*Lycopersicon esculentum* cv. Selection 22) as on 60 DAS.

Data presented in the Figures are mean ± SE scored after 60 days from 10 plants per treatment and experiment repeated thrice. Mean followed by same letters are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test.



: Impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* (T-3) treatment on morphology in leaves of tomato (*Lycopersicon esculentum* cv. Selection 22) as on 60 DAS.

Secondly, carotenoids react with chlorophyll triplets to produce carotenoid triplets and this effectively prevents the generation of singlet oxygen and also reduces life time of chlorophyll triplet which is the major photo-protective mechanism. This requires the carotenoid and chlorophyll molecules to be arranged precisely in very close proximity to each other. Both pigments are attached to the same protein forming a complex called photosynthin (Datta, 2003). Carotenoids absorb light in the blue region of the spectrum (400 to 600 nm), and the energy absorbed can be transferred to chlorophylls. Therefore, carotenoids serve as accessory pigments by harvesting radiant light in a region of the spectrum not covered by the chlorophylls. In addition, carotenoids are essential for photo-protection. In the absence of colored carotenoids,

plants suffer severe photo-oxidative damage, which generally results in the death of the organism. The likely mechanism for photo-protection is the quenching of chlorophyll triplets by colored carotenoids that would otherwise lead to the generation of oxygen singlet that can react with lipids, proteins, and other macromolecules, causing irreparable damage (Krinsky, 1979 and Davidson and Cogdell, 1981). There is considerable evidence in support of a photo-protective role of the xanthophyll cycle in the removal of excess excitation energy from the photosynthetic antennae[36].

Table 3 shows the impact of conventional farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on bio-organics in leaves of tomato as at 60 DAS. In the present investigation, there was increase in soluble proteins, total carbohydrates and polyphenol content with T_1 (67 %, 57 % and 129 % respectively), with T_2 (67 %, 19 % and 113 % respectively) and with T_3 (74 %, 55 % and 197 % respectively) compared to the control. Protein synthesis turnover in growing plants is a basic component of metabolic regulation which provides a way for varying the enzymatic complement during the response to environmental conditions[37]. Protein and carbohydrate content increased in all treatments compared to the control. It showed maximum content in T-3 treated plants. All the functions of life depend upon protein. The significantly increase in soluble protein content in the present investigation is well related to increase in photosynthetic pigment content leading to increase in photosynthetic ability of plant (Neff *et al.*, 2003). According to Ferrari *et al*. (1994), protein is the antioxidant group which protects the plant from stress induced free radical formation. So, the enhanced soluble protein content in T-3 plants in present investigation might be contributing to enhanced growth and yield. Carbohydrates are involved in structural organization of many tissues in plants. Both proteins and carbohydrates are the chief sources of energy in the living cells and are involved in ATP synthesis through oxidation process. The oxidation also produces several important intermediate compounds, which serve as carbon sources for the synthesis of amino acids, lipids and other important bio-molecules. The increase in biochemical constituents might be helpful to improve growth and yield. The term "phenolic" or "polyphenol" can be precisely defined chemically as a substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituents, including functional derivatives (esters, methyl ethers, glycosides, etc.) as a general rule. The phenolics and polyphenols arise biogenetically from the shikimate-phenylpropanoids-flavonoids pathways, producing monomeric and polymeric phenols and polyphenols (Harborne,

1989). Vincenzo *et al.* (2006) reported that plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. In the present investigation, as compared to control, the proline and glycine betaine showed highest increase with T-3 (69 % and 414 %) followed by T-1 (23 % and 274 %) and T-2 (46 % and 180 %) respectively. Proline has multiple functions, such as osmotic pressure regulation, protection of membrane integrity, stabilization of enzymes/proteins, maintenance of appropriate NADP+/NADPH ratios and scavenger of free radicals (Tripathi *et al*, 2007., Kaymakanova and Stoeva, 2008 and Misra and Saxena , 2009) and as a major source of energy and nitrogen during immediate post-stress metabolism, thereby inducing salinity tolerance Jain *et al*. (2001). Over-accumulation of proline under either salt stress or antioxidant application or their interactions in plants, has been attributed to the strategies adapted by plants to cope with stress conditions (Alqurainy, 2007). Many authors indicate the importance of soluble carbohydrates in stimulating proline accumulation through an inhibition of the degradation enzymes of proline (Heineke *et al*., 1992) and synthesis of enzymes of proline formation. Glycine betaine (GlyBet), a quaternary ammonium compound, is regarded as one of the most effective osmoprotectants owing to its many advantages besides its efficacy as a compatible solute. The molecular features of GlyBet enable its interaction with both the hydrophobic and hydrophilic domains of macromolecules without perturbing the cellular functions (Sakamoto and Murata, 2000). Ma *et al.* (2004) have also reported that GlyBet induced the accumulation of osmolytes, such as soluble sugars and free proline. In the present study, ascorbic acid content increased significantly by 33 % with T-1, by 15 % with T-2 and by 37 % with T-3 respectively compared to the control. Horeman *et al.* (2000) stated that ascorbic acid is involved in other functions such as plant growth, gene regulation, and modulation of some enzymes and redox regulation of membrane –bound antioxidant compounds.

Figure 1 depicts the impact of NPK farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on oxidative enzyme activity in leaves of tomato as at 60 DAS. Enzymatic activity is correlated with cell division and cell differentiation at various stages of leaf development (Maksymowych and Kettrick, 1970). In the present investigation, as compared to control, there was increase in the activity of catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), IAA Oxidase (IAO) and super oxide dismutase (SOD) under T-1 (1.5 %, 33.33 % , -43% , 29 % and 5.% respectively), T-2 (0.08 %, 75 %, 71 %, 9.5% and 56 % respectively)

and T-3 (12 %, 83 %, 100 %, 45 % and 70.5 % respectively). Gogorcena *et al.* (1995) and Bergmann *et al.* (1999) reported that antioxidative enzymes were related with water deficiency and they were considered the main components of anti-oxidative machinery for drought resistance in higher plants. According to Shigeoka *et al.* (2002), peroxidase catalyses the dehydrogenation of structurally diverse phenolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes. Sen and Mukharji (2009) reported that IAA oxidase controls IAA levels in plants and is hence responsible for regulating growth. The present study shows that T-3 led to greater increase in activity of polyphenol oxidase and IAA oxidase compared to the peroxidase and superoxide dismutase enzyme activity. This led to an increase in anti-oxidant properties of tomato plant, which was significant. Djianaguiraman *et al.* (2005) concluded that SOD activity and the removal of H_2O_2 by catalase and peroxidase are necessary for an effective defense against the action of free radicals. SOD plays an important role in protecting cells against the toxic effects of superoxide radicals produced during oxidative burst (Halliwell and Gutteridge, 2000). In the present investigation, highest activity of super oxide dismutase was observed in T-3 plants.

CONCLUSION

The present investigation showed that the number of leaves per plant, leaf area and biomass were highest in T-3, while leaf area was same in T-1 and T-3. Membrane injury was lowest in T-3 followed T-1 and T-2. Cholorophyll content showed nearly same increase in T-3 and T-1 over T-2. Chlorophyll stability index and xanthophylls were highest in T-3, followed by T-1 and T-2. There was significant increase in soluble proteins, total carbohydreates, polyphenols, ascorbic acid, proline and glycine betaine under T-3 keeping T-1 in second rank. T-3 acquired first position in activity of oxidative enzymes such as catalase, peroxidase, polyphenol oxidase, IAA oxidase and super oxide dismutase.

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