Influence of *kunapajala* treatment from *vrikshyaurveda* on leaves of tomato (*lycopersicon esculentum* 1. Cv. Selection 22) and its comparison with conventional farming and organic farming.

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Abstract—Kunapajala is a fermentation product of easily available ingredients and it works as a natural plant growth booster. The experiments were conducted using pot culture method for conventional 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. The leaf area was same under both conventional farming and kunapajala treatment. The 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 conventional farming and organic farming. Bioorganic 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 conventional 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 conventional farming and organic farming and physiological, biochemical and enzymatic enhancement in the leaves of tomato under organic farming conditions.

Keywords—Bio-organics, kunapajala, leaf, oxidative enzymes, organic farming, tomato.

Abbreviations

- 1. T-1 = Conventional farming
- 2. T-2 = Organic farming
- 3. T-3 = Kunapajala treatment
- 4. g = gram
- 5. mg = milligram
- 6. Chl = Chlorophyll
- 7. CSI = Chlorophyll stability index
- 8. RWC = Relative water content
- 9. O.P. = Osmotic potential
- 10. DAS = Days after sowing
- 11. ATP = Adenosine triphosphate
- 12. D.W. = Distilled water
- 13. GlyBet = Glycine betaine
- 14. NADPH = Nicotinamide adenine dinucleotide phosphate
- 15. POD = Peroxidase
- 16. PPO = Polyphenol oxidase
- 17. SOD = Super oxide dismutase
- 18. min= Minute
- 19. Δ O. D. = Change in optical density

I. INTRODUCTION

The vegetables are important in human diet for their vitamin and mineral content essential for metabolic processes taking place within the human body. Tomato is solanaceous fruit vegetable available round the year. It is cultivated in tropics and subtropics of the world. It is believed that consumption of one tomato per day enhances the health status of individuals and it is considered to be important in diet as it is quite high in nutritive value¹. 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 agriculture production in future. The cost of fertilizers has been enormously increasing to an extent that they are 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 the 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 the 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 crop with better nutrient quality. Organic food (Green food) catches 2 to 5 times more market price². Unfortunately the productivity of organically grown crop is less.

Liquid bio-fertilizers play a vital role in organic farming leading to green food production which is safer, healthier and tastier. The concept of bio-fertilizer is mentioned in Vrikshyaurveda under the generic name 'kunapajala' by Surpala (1000 AD) in eastern India³. Kunapajala is a fermentation product using easily available ingredients like Sesamum indicum L. (Tila), bone marrow, flesh (sheep, goat, fish etc), milk, black gram (Vigna mungo), ghee, honey etc. The beauty of kunapajala is that, it can be used on any plant at any growth stage. Firminger⁴ mentioned the beneficial use of liquid manure kunapajala for vegetable cultivation. According to Neff et al.⁵ (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, Patil⁶ (2007) mentioned that there is always a danger of passing on dormant pathogen to fields with plant based compost. But this is avoided by kunapaiala 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 condition⁷. Nene⁸ 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. Mishra⁹ 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 Naik¹⁰ 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 way.

To address this short fall, 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 conventional farming and organic farming.

II. METHODOLOGY

The experiments were conducted at P.G. Research Centre, Department of Botany, Tuljaram Chaturachand College, Baramati, Dist. Pune, (M.S.) India (between $18^{\circ}3'$ N to $18^{\circ}12'$ latitude, $74^{\circ}13'$ E to $74^{\circ}40'$ E longitude and 548 m above mean sea level), in shade house using pot culture method. The earthen pots (40×40 cm) were used for the experimentation. Conventional 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.*⁷ 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. 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.*¹¹ and Premchandra *et al.*¹² respectively. The photosynthetic pigments like chlorophylls and carotinoids were estimated by methods proposed by Arnon¹³ and Jensen¹⁴ respectively. The biochemical constituents were analyzed using the methods proposed by Lowry *et al.*¹⁵ for soluble proteins, Sadasivam and Manikam¹⁶ for total carbohydrates and for ascorbic acid, Grieve *et al.*¹⁷ for glycine betaine and Bates *et al.*¹⁸ for proline. The enzyme catalase was assayed according to the method described by Luck¹⁹. The activity of peroxidase enzyme was determined according to the method of Malik and Singh²⁰ and that of polyphynol oxidase by Mahadevan and Shridhar²¹. Activities of super oxide dismutase and IAA oxidase were analyzed using the methods proposed by Giannopolitis and Ries²² and Tang and Bonner²³ respectively.

III. RESULTS AND DISCUSSION

Impact of conventional farming (T-1), organic farming (T-2) and kunapaiala treatment (T-3) on morphological parameter and water relations in leaf of tomato as on 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 showing the impact of conventional farming (T-1), organic farming (T-2) and kunapajala (T-3) treatment on morphology in leaves of tomato (Lycopersicon esculentum Mill. cv. Selection 22) as on 60 DAS. Plant size and leaf area are important variables in breeding for crop adaptation to water-limited environments. Singh et al.²⁴ 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 increase 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.²⁵ and Singh et al.²⁶ remarked that the attainment of biomass was significantly and positively correlated with seed yield. The T-3 treatment 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 Ludlow²⁷ reported that, plant metabolism is dependent on leaf water status. RWC has been proposed as a selection criteria for drought tolerance in many crops as reported by Schonfeld *et al.*²⁸ in barley and Martin *et al.*²⁹ in wheat. In the present study values of RWC and OP were highest in T-3 followed by T-1 and T-2 which play 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 conventional farming (T-1), organic farming (T-2) and kunapajala treatment (T-3) on photosynthetic pigments in leaves of tomato as on 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, the chlorophyll a showed nearly same increase with T-1 and T-3 by 49 % followed by T-2 (29 %) treatment. However, chlorophyll b showed more increase with 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 $^{30, 31}$. 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 production³². They react with singlet oxygen to produce carotenoid triplet which then decays harmlessly producing heat, rather than any toxic product. 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 Photosynthin³³. 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^{34, 35}. There is considerable evidence in support of a photoprotective role of the xanthophyll cycle in the removal of excess excitation energy from the photosynthetic antennae³⁶.

Table 3 exhibits the impact of conventional farming (T-1), organic farming (T-2) and kunapajala treatment (T-3) on bio-organics in leaves of tomato as on 60 DAS. In 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 conditions³⁷. 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 plant⁶. According to Ferrari *et al.*³⁸, 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 polyphenols³⁹. Vincenzo et al.⁴⁰ 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 increase highest 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^{41,42,43} and as a major source of energy and nitrogen during immediate post-stress metabolism, thereby inducing salinity tolerance⁴⁴. 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 up with stress conditions⁴⁵. Many authors indicate the importance of soluble carbohydrates in stimulating proline accumulation through an inhibition of the degradation enzymes of proline⁴⁶ 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 functions⁴⁷. Ma *et al.*⁴⁸ have also reported that GlyBet induced the accumulation of osmolytes, such as soluble sugars and free proline. In 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.⁴⁹ 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 represents the impact of conventional farming (T-1), organic farming (T-2) and *kunapajala* treatment (T-3) on oxidative enzyme activity in leaves of tomato as on 60 DAS. Enzymatic activity is correlated with cell division and cell differentiation at various stages of leaf development⁵⁰. 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.*⁵¹ and Bergmann *et al.*⁵² 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.*⁵³, peroxidase catalyses the dehydrogenation of structurally diverse phenolic substrates by H₂O₂ and are thus often regarded as antioxidant enzymes. Sen and Mukharji⁵⁴ reported that IAA oxidase controls IAA levels in plants and is hence responsible for regulating growth. The present study shows that T-3 leads to more increase in activity of polyphenol oxidase and IAA oxidase compared to the peroxidase and superoxide dismutase enzyme activity. This leads to increase in antioxidant properties of tomato plant, which is significant. Djianaguiraman *et al.*⁵⁵ concluded that SOD activity and the removal of H₂O₂ by catalase and peroxidase are necessary for an effective defense against the action of free radicals. SOD plays an important role in protecting cell against the toxic effects of superoxide radicals produced

during oxidative burst⁵⁵. In the present investigation, highest activity of super oxide dismutase was observed in T-3 plants.

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

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

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

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

Sr.	Parameters	Control	Tre	atments	
No.			Conventional 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	59.55 ^c	72.78 ^{a,b}	65.49 ^b	73.23 ^a
	(mg / g fresh wt.)	±0.90	±1.154	±1.42	±1.12
03	Total chlorophylls	139.1 ^c	191.56^{a}	167.84 ^b	191.96 ^a
	(mg / g fresh wt.)	±1.34	±1.78	±1.25	±1.13
04	Chlorophyll stability Index	0.57	0.87	0.92	0.95
05	Carotenoids	19.91 ^c	21.01 ^{a,b}	20.37 ^b	21.14 ^a
	(mg/ 100g)	±0.33	±0.56	±0.35	±0.47
06	Xanthophylls	4.32 ^d	4.72 ^c	6.56 ^b	7.28^{a}
	(mg/ 100g)	±0.29	±0.63	±0.13	±0.47

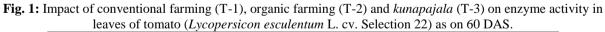
Data presented in the table are mean \pm SE scored after 60 days from 10 plants per treatment and experiment repeated thrice. Mean followed by same letters are not significantly different at $P \le 0.05$ level by Duncan's multiple range test.

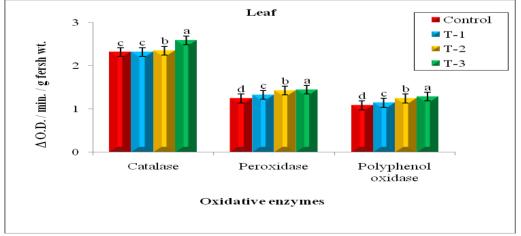
Sr. No.	Parameters	Control	Treatments		
INO.			Conventional farming (T - 1)	Organic farming (T - 2)	Kunapajala (T - 3)
01	Soluble proteins (g 100 ⁻¹ g fresh wt.)	7.60 ^c ±0.66	12.7 ^b ±0.11	12.7 ^b ±0.33	13.2 ^a ±0.1
02	Total carbohydrates (g 100 ⁻¹ g fresh wt.)	12.1 ^d ±0.22	19.0 ^a ±0.5	14.44 ^c ±0.22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

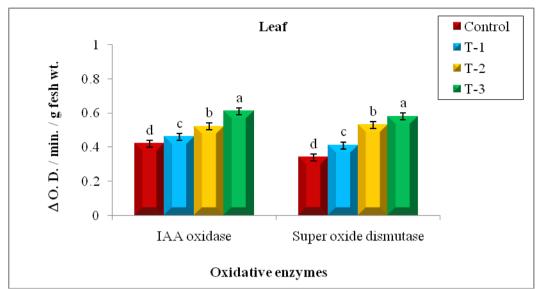
Table - 3: Impact of conventional farming (T-1), organic farming (T-2) and kunapajala treatment (T-3) on bio-
organics in leaves of tomato (Lycopersicon esculentum L. cv. Selection 22) as on 60 DAS.

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

FIGURE







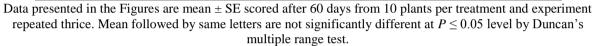


PHOTO PLATE

