Date: 20/11/2015

To,

The Principal,
Sapthagiri College of Engineering,
Bangalore-560057,

Through HOD & RDECI

From

Shobha G,
Departmentof Biotechnology
Sapthagiri Collegeof Engineering
Bangalore-560057

Respected Sir,

Subject: Requisition for the financial assistance for carrying out research project

With respect to above subject, I request your kind self to provide finical assistance to carry out the project entitled "**Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant**". The research proposal and budget split up has enclosed along with this letter. Kindly consider the requisition and do the need full.

Thanking you

Your's sincerely

RDECI :

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesareghatta Road,
Bangalore-560 057

12

RESEARCH PROPOSAL

A. GENERAL INFORMATION

1	**About the project**		
a	Title of the project	:	Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant
b	Subject area as per instruction	:	Biological & Agricultural Sciences
2	**Details of Principal Investigator**		
a	Name	:	MrsShobha G And MrsSoumya C
b	Qualification	:	M.Sc, M.Phil, (Ph.D)
c	Designation	:	Assistant Professor
d	Department	:	Biotechnology
e	Years of teaching/research experience	:	08& 11
f	Email ID	:	shobhag@sapthagiri.edu.in
g	Cell Number	:	9964591024
h	**Details of the Head of the Department**		
i	Name of the Head of the Department		Dr. Ananda S
j	Email ID	:	hodbt@sapthagiri.edu.in
k	Cell Number	:	9900833873

Signature of the Investigator

Signature of Head of the Department

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

A. DETAILS OF THE PROJECT PROPOSAL

1.	**Title of the Project Proposal:**
	Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant
2.	**Objectives of the proposal:**
	• Assessment of antioxidant enzyme SOD, Peroxidase, GPX, Catalase, in tomato plant by invitro method • Assessment of antioxidant properties in tomato plant by invitro method
3.	**Background of the project:**
	The innovation relies on copper, a red metal valued for centuries for its antibiotic properties and used in potting soil. Copper a microelement required for the development of plant and electron transferring enzymes, deficiency have showed leaves curled, and their petioles bend downward, chlorosis with loss of turgor in the young leaves. *Lycopersiconesculentum*, the second largest crop in global agricultural production, and the second producer in India. Diagnosis to detect copper deficiency in plants is an extremely important management tool because copper deficiency can produce devastating yield losses, often with little evidence of the characteristic symptoms. However a Cu concentration higher than 5 mg has showed toxicity in uptake of nutrients. Hence more research is needed to understand possible long-term impacts of nanotechnology in agriculture. **Thus, the goal was to gain a better understanding regarding the Antioxidant Enzyme and Antioxidant Propertiesof Tomato Plant against copper nanoparticle.Ultimately to develop recommended exposure limits for the plant development.**
4.	**Methodology :**
	1. Antioxidant Enzyme Assays The antioxidants enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activity were studied. **Preparation of Enzyme Extract** **Super Oxide Dismutase (SOD: EC 1.15.1.1)** The total 2.6ml volume reaction mixture of was made up of 0.1ml of extract, 0.5ml of sodium

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Bangalore-560 057

phosphate buffer, 1ml of Na_2CO_3, 0.4ml of NBT and 0.2ml of EDTA. The response was started by including 0.4ml of $H_3NO \cdot HCl$and exposing reaction mixture to white light for 15min at RT. The absorbance was measured at 560nm using spectrophotometer. The SOD activity is defined as the amount of photochemical reduction of NBT in one minute using molar extinction coefficient of 4020 M^{-1} cm^{-1}[Mirsa and Fridovich 1977 and Kono 1978].

Glutathione Peroxidase (GPX: EC1.6.4.2)

The activity of enzyme was measured as per Mohandas et al [Mohandas et al 1984]. The total 2ml reaction volume contained sodium phosphate buffer, EDTA, sodium azide, 0.1 ml of enzyme extract, glutathione, NADPH, 0.1ml of PMS and H_2O_2. The disappearance of NADPH at 340nm was recorded. The enzyme activity was calculated using molar extinction coefficient of 6220 M^{-1} cm^{-1}.

Catalase (CAT: EC 1.11.1.6)

The CAT activity was assayed as described by Aebi [Aebi1984], the reaction volume consist of 1.5ml sodium phosphate buffer, 0.5ml H_2O_2 and 0.1ml of enzyme extract. The volume is made to 3ml by adding dH_2O. The activity was estimated by the reduction in absorbance at 240nm against blank phosphate buffer and unit was calculated using molar extinction coefficient of 43. 6 M^{-1} cm^{-1}.

Polyphenol Oxidase (PPO: EC 1.10.3.1)

According to Mahadevan and Sridhar [Mahadevan and Sridhar 1982] protocol, the activity of enzyme was measured using catechol as a substrate. The reaction volume consists of 3.0ml of sodium phosphate buffer, 1.0ml of catechol in the buffer and 0.5ml of enzyme extract. Increase in absorbance was measured at 495nm. Activity was estimated using a molar absorption of 1010 M cm^{-1}.

Phenyl Alanine Aminolyase (PAL: EC 4.3.1.24)

The reaction volume contains 0.1ml enzyme extract with 0.5ml of sodium phosphate buffer and 0.2ml of L-phenylalanine and distilled water was added to make the final volume 3ml. PAL activity was measured by an increase in the absorbance at 290nm against blank phosphate buffer and units was calculated using a molar absorption at the rate of 19.73 mM^{-1} cm^{-1} [Goldson et al 2008].

Soluble Proteins

Lowery et al [Lowery et al 1951] method was followed for protein estimation using BSA as a standard. In brief, 0.2ml of protein extract was incubated for 10min at RT in dark condition after addition of 5ml alkaline copper solution followed by addition of 0.5ml FC reagents. The tubes were vortexed and

allowed to stand for 30min at room temperature. The absorbance was recorded at 660nm against blank without protein extract.

2. Antioxidant Properties Assays

Determination of Malondialdehyde (MDA) content

Seedlings/Fresh leaf (500 mg) of treated and untreated control tomato plants were homogenized in 6ml TCA. The homogenate was centrifuged at 10,000rpm for 10min. The supernatant was used as a sample for analysis. For 2ml extract, 2ml of 0.6% TBA solution prepared in 20% TCA was added. The reaction mixture was mixed thoroughly and placed in the boiling water for 30min and cooled rapidly, followed by centrifugation for 15min at 10,000rpm. The reaction mixture was read at 532nm, 450nm and 600nm against TCA blank. The MDA concentration was determined by its molar extinction coefficient of 155 mM/cm and expressed as nmoles/ml of fresh weight [MadhavaRao and Stresty, 2000].

$$A = [(Abs532+TBA) - (Abs600+TBA) - (Abs532-TBA-Abs600-TBA)]$$

$$B = [(Abs440+TBA-Abs600+TBA) \times 0.0571\}$$

$$\text{MDA equivalents (nmol/ml)} = (A-B/157000) \times 106$$

Determination of Free Radical Scavenging Activity by Diphenylpicrylhydrazyl method

The assay was conducted according to Lee et al [Lee et al 1981] with some adjustments, according to which the 0.5ml of methanolic extracts of seedlings/leaf and 1.5ml of DPPH methanol solution was taken and allowed to stand in dark for 30min at room temperature. The absorbance was recorded at 515nm against methanol blank.

Determination of Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method

Briefly, 0.5ml of the seedling/leaf extract was mixed with a 3ml phosphomolybdic acid reagent solution. The reaction mixture is then incubated in water bath at 95°C for 90min. After cooling, the absorbance of reaction mixture was measured at 695nm against methanol blank [Prieto et al1999]. The TAC was expressed as the number of gram equivalent of ascorbic acid (GAE) per gram of fresh weight.

5.	**Milestones with time schedule & work plan: 6-12 Months**		
6.	**List of equipment required for Phase-I & Phase-II for Project Implementation**		UV-Spectrometer Kits Rs.3,00,000 approximately
7.	**Relevance, importance & application of the project:**		
	Researchers are now working to bridge a gap between agriculture and nanotechnology, so that of agri-nanotechnology can revolutionize the sector with new tools for disease detection, targeted treatment,		

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	enhancing the ability of plants to absorb nutrients, fight diseases, delivering nutrients or pesticides as appropriate, improve our understanding of the biology of different crops and thus potentially enhance yields. **At a same time nanoparticles and their increased use have raised concerns about their possible harmful effects within the environment.**		
10.	**Novelty/Uniqueness of the project proposal**	:	Over time, the results explains, nanomaterial in the agricultural inputs which may shows the positive and negative impact on growth of *Lycopersiconesculentum* and may contribute to the controversial debate on plant toxicity of nanoparticles.

Name & Signature of the Principal Investigator (with seal)

Name & Signature of Head of the Department (with seal)

Principal
Sapthagiri College of Engineering
14/6, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

Date: 11/01/2016

From,

Research and Development cell,
Sapthagiri College of Engineering,
Bangalore-560057.

Through: HOD

To,

Principal investigator,
Sapthagiri College of Engineering,
Bangalore-560057.

Subject: Sanction of research grants Reg.,

The committee hereby informed that following project have been approved for the academic year 2015. The report and the outcome of the project have to be submitted to the committee after the completion of the project. The utilization certificate shall be given along with the final report.

Sl. No	Principal Investigator	Department	Project entitled	Amount Sanctioned
1	MrsShobha G/ MrsSoumya C	BT	Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant	3,00,000

Convener

Copy To,

Principal
All Departments
IQSC

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

Date :25/01/2016

To,

Principal,
Sapthagiri Collegeof Engineering
Bangalore-560057

Through HOD

From,

Shobha G,
Department of Biotechnology,
Sapthagiri Collegeof Engineering
Bangalore-560057

Respected Sir,

Sub: Procurement of Instruments Reg.,

With reference to the letter received from R& D committee regarding approval of research project, we are here by requesting the procurement of the InstrumentsPC based double beam UV-VIS Spectrophotometer for the research project entitled "**Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant**" to carry out. This will enhance the research potential to contribute for the improvement in the field of biotechnology.

The instrument cost around **Rs 3, 00, 000/- (Three lakh fifty thousand).** Kindly consider the request and do the need full.

Thanking you,

Yours Faithfully,

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Date: 25/03/2016

To,

Research and Development cell,
Sapthagiri College of Engineering
Bangalore-560057

Through HOD

From

Shobha G,
Departmentof Biotechnology
Sapthagiri College of Engineering
Bangalore-560057

Sub: **Submission of report and utilization details**

With reference above cited subjected I am hereby enclosing project report entitled "**Study of Antioxidant Enzyme and Antioxidant Properties of Tomato Plant**" and utilization certificate. This for your kind information, please.

Sl No	Particulars	Quantity	Amount	Remarks
1	PC Based Double Beam UV-VIS Spectrophotometer	1	284625/-	Purchased

Thanking You,

Copy,

Principal

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Principal
Sapthagiri College of Engineering
Chikkasandra, Hesaraghatta Road,
Bangalore-560 057

STUDY OF ANTIOXIDANT ENZYME AND ANTIOXIDANT PROPERTIES OF TOMATO PLANT

Submitted By

Mrs. Shobha G and Mrs Soumya C

Assistant Professor

Department of Biotechnology

To

SAPTHAGIRI
College of Engineering
Creating Tomorrow

DEPARTMENT OF BIOTECHNOLOGY

SAPTHAGIRI COLLEGE OF ENGINEERING

BENGALURU-560057

KARNATAKA, INDIA

2016-2017

INTRODUCTION

The green revolution in the agriculture sector in past decades has resulted in a great increase in the quantity and quality of agricultural products leading into the transition from traditional agriculture to commercial agriculture and the success was accompanied with the overuse of agricultural resources, in spite of all this the global food shortage is not yet resolved. According to the United Nations, about 800 million people in the world are suffering from a shortage of food and number of people below the poverty line who are unable to afford food has dramatically increased [ZenuJhaet al 2011]. This is due to the gap that exists between potential yield of crop and final yield observed in farmer's plots which is largely owed too many abiotic and biotic stress causes in field affecting the crop [JaggalSomappaet al 2013]. Every year there is a significant crop yield loss due to nutritional deficiency in soil and devastating diseases worldwide [Wani 2011]. Scientific study over the years is trying to fill the gap, between engineering of agriculture and science with innovative science-based technologies and the nanotechnology makes the best answer here. Many fields like materials science, electronics and medicine, have seen the considerable use of nanotechnology and from past few decades since they applied nanotechnology in research. However, unlike researchers from other disciplines, it is only during recent years that we have started to see the potential applications of nano-science in agriculture [Eduardo Corredor et al 2009].

Agriculture system can be revolutionized by the use of nanotechnology in various ways. Engineered nanomaterial has several applications in agriculture field such as induction of faster germination of seed, crop production, crop protection from pests and diseases, nutrition and water supply management, disease treatment delivery system, new material for pathogen detection, the modern tools for molecular biology, protection of environment, delivering nutrients and hence in turn enhancing the yields or nutritional values potentially [Remya Nair et al 2010]. In addition to this, it can also offer directions for environmental remediation or adds value to crops, thereby our understanding of the crop biology can be improved. Nanoparticles (NPs) commonly used in the agricultural field fall into the following categories viz., metal, metal oxide nanoparticles, and non-metal nanoparticles, all belonging to the family of nanomaterials. The nanomaterials are nowadays receiving increasing attention in a vast variety of field as it is transitional between individual molecules and bulk materials and hence exhibit unique optical, electronic, magnetic properties that which otherwise in their bulk forms, they normally will not display [Nelet al 2006, Ruffini Castiglione Monica et al 2009, Elena Masarovičová and KatarínaKráľová et al 2013, Srivastava and Dwivedi KN, 2012]. These nanoparticles are in various shapes such as nano-powder, nano-wires, nano-cluster or nano-crystals and are of different sizes varying from 1nm to 100nm ranges. It has been reported that both favourable and non-favourable influence on crop rely upon the properties of nanomaterials for example surface area,

diameter, reactivity and concentration of NPs and its uptake, translocation, and transportation by the plants to different tissues, and also the type of plant [Jie Yang et al 2013].

MATERIALS AND METHODS

Antioxidant Enzyme

The antioxidants enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activity were studied.

Preparation of Enzyme Extract

The 5ml of 50mM sodium phosphate buffer (pH 7) containing 5mM EDTA and chilled mortar with pestle was used to create homogenized seedlings/ fresh leaf (500 mg) extract of treated and untreated control samples. The homogenate was centrifuged at 10,000rpm for 20min at 4^0C and supernatant was collected. The residues were re-extracted with 5ml buffer, the two supernatant were merged and used for the enzyme assay. The enzyme activity was measured by recording absorbance at particular absorbance for 1min and was expressed as µM/min/mg of protein (Activity (IU)/mg of protein).

Super Oxide Dismutase (SOD: EC 1.15.1.1)

Principle

The radical formed will be detoxified by the enzyme which converts more toxic superoxide anion radicals into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2). The superoxide ion (O_2-) converts Nitrobluetetrazolium (NBT) to NBT-diformazan, which absorbs light at 560nm.

Reagents

Sodium phosphate buffer (50mM, pH 7.6), 125mM Sodium carbonate, 25µM Nitrobluetetrazolium, 0.1mM Ethylene diamine tetra acetic acid, 1mM Hydroxylamine hydrochloride

Procedure

The total 2.6ml volume reaction mixture of was made up of 0.1ml of extract, 0.5ml of sodium phosphate buffer, 1ml of Na_2CO_3, 0.4ml of NBT and 0.2ml of EDTA. The response was started by including 0.4ml of $H_3NO{\cdot}HCl$and exposing reaction mixture to white light for 15min at RT. The absorbance was measured at 560nm using spectrophotometer. The SOD activity is defined as the amount of photochemical reduction of NBT in one minute using molar extinction coefficient of 4020 M^{-1} cm^{-1}[Mirsa and Fridovich 1977 and Kono 1978].

Glutathione Peroxidase (GPX: EC1.6.4.2)

Principle

Invoice-Cum-Challan for Removable Goods from Factory or Warehouse on payment of duty (Rule 11 of Central Excise (No.2) Rule, 2001

SYSTRONICS (INDIA) LIMITED

SYSTRONICS Division, An ISO 9001 : 2008 Co.

89-92, Naroda Industrial Area, Naroda, Ahmedabad - 382 330.
Phone : 79-2281 3017, 2281 3117 Fax : 91-79-2281-1592.

TIN No. 24073101396 Dated 31/08/2007
CST No. 24573101396 Dated 31/08/2007

Name of Excisable Commodity :
Scientific & Electronics Analytical Instruments and Test & Measuring Instruments & Oscilloscopes, Parts & Accessories thereof.

F/SALES
REV : 00

RETAIL INVOICE

Billing Party

THE PRINCIPAL,
SAPTHAGIRI COLLEGE OF ENGINEERNG.,
CHIKKASANDRA HESARAGATTA MAIN ROAD,
BANGALORE - 560 073

Consignee Party :

THE PRINCIPAL,
SAPTHAGIRI COLLEGE OF ENGINEERNG.,
CHIKKASANDRA HESARAGATTA MAIN
BANGALORE - 560 073

Invoice No. 211611987
Invoice Date 25.02.2016

Mode of Despatch:

Despatch Date:

Frieght:
RR/AWB/GC/RRR N
No of Packages 1

Item Type/ Code	Item Description	PO NO: SSCET:HO:4730COMP:84 PO DT: 23/02/2016	QTY	Rate PER Unit	
2202	PC BASED DOUBLE BEAM UV-VIS SPECTROPHOTOMETER	Asses Rate 284,625.00 Tot. Ass. Value 284625.00	1	284,625.00	28
SR NO	,738				

ACCS
05/04/16

Forwarded for kind need full

Instrument received with good condition, Demo have taken by application specialist from systronics, and Equipment taken in stock book page No: 31,

Total Item Value 2
Less Dis @ 20% 5
Assessable Value 2

CST @ 5%

Mode of Payment
Rs: TWO LAKH THIRTY NINE THOUSAND EIGHTY FIVE ONLY

Total 2

To Pay Amount 2

Entered in stock - Instrument - P.No: 31
* Unit was also test with sample, working satisfactory

Head of the Department, Dept. of Chemistry, Sapthagiri College of Engineering, No. 57/1, Chikkasandra, Hesaraghatta Main Road, Bangalore-57

Principal, Sapthagiri College of Engineering, Chikkasandra, Hesaraghatta Road, Bangalore-560 057

Pre authenticated
For Systronics (India) Limited
Systronics Division

Principal, Sapthagiri College of Engineering, 14/5, Chikkasandra, Hesaraghatta Main Road, Bengaluru - 560 057

Authorised Signatory

Office of Asstt. Commissioner,
Central Excise Div. 1 Jivabhai Mansasion
Ashram Road, Ahmedabad-9.
Range Office : Range III, Divn. I
Jivabhai Chambers,
Ashram Road, Ahmedabad-380 009.
Commissionerate : Ahmedabad-II
E.C.C. / Reg. No. AADCS2709JEM001

E.C.C. / Reg. No. AADCS2709JEM001
Monthly payment scheme as per Rule 8 of C.E. (No.2) Rules, 2001
Date and Time of Issue of Invoice-Cum Challan :

Date & Time of Removal of Goods

2/4/15

CERTIFICATE
Certified that the particu above are true and correc amount indicated represents actually charged and that t flow additional consideration indirectly from the buyer
For Systronics (India) Limi
Systronics Division
Authorised Signator

Regd. Office : B/116-129, Supath II Complex, 1st Floor, Nr. Juna Wadaj Bus Turminus, Ashram Road, Ahmedabad-380 013.

SRI SRINIVASA EDUCATIONAL & CHARITABLE TRUST (R)

SAPTHAGIRI COLLEGE OF ENGINEERING

(Affiliated to Visveswaraya Technological University, Belgaum & Approved by AICTE - New Delhi)

UTILIZATION CERTIFICATE

Sl No	Particulars	Quantity	Amount
1	UV spectrometer		2,84,625
		Total	2,84,625

Certified that Sapthagiri college of Engineering has provided partial financial support of **RS 2, 84,625/-**(Two lakh eighty four thousand six hundred twenty five only) towardsStudy of Anti-Oxidant Enzyme and Anti-Oxidant Properties of Tomato Plantproject by BiotechnologyDepartment of Sapthagiri college of Engineering.

Certified that I have satisfied myself that condition on which the grant in aid sanctioned has been duly fulfilled and that I have excised the following check to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of check exercised

1. Bills

Signature of the Principal with seal

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057

Signature of Auditor with seal

M A S & Co CHARTERED ACCOUNTANTS VERIFIED, BANGALORE 28/08/2020

Principal
Sapthagiri College of Engineering
14/5, Chikkasandra, Hesaraghatta Main Road
Bengaluru - 560 057