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ANTIOXIDANTS AND TYPE – II DIABETES RELEVANT ENZYME INHIBITION OF MOTH BEANS (*Vigna aconitifolia*) GERMINATED WITH ASCORBIC ACID AS AN ELICITOR

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ABSTRACT

This study aims to determine the effect of elicitor on the antioxidant, α- amylase and α- glucosidase inhibition activity of germinated moth beans. The germination was carried out by hydrating the seeds in varying concentration (0-200mg/L) of ascorbic acid. The results showed that the Total antioxidant capacity (TAC) as well as α- amylase and α- glucosidase inhibition activity was increased significantly (P ≤ 0.05) in the germinated samples. The total phenolics, Flavonoid and Proline levels were increased significantly (P ≤ 0.05) in the germinated samples compared to the raw moth beans.Flavonoid, DPPH–RSA and ABTS-RSA also increased in germinated moth beans. The carbohydrate digestive enzymes (α-amylase and α-glucosidase) activity was inhibited significantly in germinated moth beans. The Pearson correlation showed a significant relation between Total phenols and TAC. The findings suggested that the moth beans sprouts may be used as a nutraceutical food with the potential to give the quality of human health and alleviate the effect of prevalent of non-communicable disease.

***Key Words**: Moth beans, Elicitor, Antioxidant, Diabetes mellitus, Ascorbic acid*

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes (T2-D)with its rapid increasing prevalence become a global public health problem. The pathogenesis and progression of this disease as well as development of secondary complications of T2-D is directly linked to chronic hyper-glycemia and oxidative stress [1].Therefor regulation of blood sugar level after meal by modulating the activities of carbohydrate digesting enzymes such as α – amylase and α – glucosidase is main strategy for controlling chronic hyper-glycemia [2]. Current treatment to control hyper glycemia is the use of synthetic inhibitors to regulate the activity of these enzymes, and to delay the rate of glucose absorption in small intestine [3]. These synthetic drugs inhibit the enzyme but may cause severe side effects such as gastrointestinal distress and hence, search for safe hypoglycemic agents from plant foods which are commonly consumed using in vitro models has advantage [4].

Plant foods containing varieties carbohydrate digestive enzymes namely α-amylase and α-glucosidase and are not showing any side effect [5]. These compounds are also considered as antioxidants therefore, they may provide additional protection against oxidative stress induced by hyperglycemia. The phytochemical composition of plant foods depends on genetics, physiological and agronomical factors which are known as abiotic and abiotic and can be used to increase phenolic compounds in plant foods. These health's related phenolic compounds can be increased in plant systems by stimulating protective endogenous pathways such as redox-linked pentose phosphate pathway (PPP) [6].

Eliciation is a process in which elicitor application can be used to increase metabolite productionin plant and to increase its nutraceutical value for that plant [6] .Various research studies have reported increased phenolic metabolites in legumes in response of elicitor treatment along with germination through PPP associated endogenous defence response [7,8,9]. Oregano extract, Folic acid, and Chitosan are considered as natural elicitors. Ascorbic acid is a strong water soluble antioxidant and induced a stress response in the host leading to increased synthesis of bioactive phenolics, L- DOPA and enhanced antioxidant activity [10]. Germination is a simple, low cost and home scale process which improves nutraceuticals and bioactive compounds of legumes.

MATERIALS AND METHOD

Chemicals: L-Ascorbic acid (95210), DPPH (2,2-Diphenyl – 1-picrylhydrazyl) (D 9132), Gallic acid (G7384),N-(1-napthylethylenediaminedihydrochloride) (N 9125), Rutin hydrate (R 5143), TPTZ (2,4,6-Tris (2-pyridal) -s- triazine) (T 1253) , PPA (porcine pancreatic alpha amylase type VI-B) (A 3176), Acarbose (A 8980), pNPG (4-p nitrophenyl – α- glucosidase (Type I from baker's yeast) (G 5003), were purchased from Sigma Aldrich Company (St. Louis, MO, USA). Soluble starch purchased from SRLMumbai, India. All other chemicals were purchased from local manufacturer and were AR grade.

Seed treatment and germination:

Mothbeans (*Vigna acontilifolia*) were purchased from the D- martstore of V. V. nagar, Gujarat, cleaned and stored in plastic container till further used. The seeds (50 g) were primed in ascorbic acid solution having varying concentration of ascorbic acid(0 to i.e. 0 to 200mg /L. Fifty grams of seeds were placed in 500 ml of solution in 1000ml of conical flasks. Then soaked for 12 hrs. The pre-soaked seeds were washed with distilled water and germinated at 37 ˚C for 24 hrs. between two layers of filter paper and kept moist by sprinkling water at regular time period. These sprouts were dried, powdered and stored at – 20 ˚ C till analysis. The known amount of (3 g.) of powder was extracted

using 15 ml of methanol: water (80:20) solvent with P^H 2. The dried powder was crushed in a motor and pestle. The mixture was kept in shaker for 30 minutes. The content were centrifuged for 10 minutes at 6000 rpm. The supernatant was collected in a sugar tube. To the residue 10 ml of methanol: water was added and process was repeated. The supernatant was pooled and made the volume to 50 ml with the solvent. There extracts were analyzed for biochemical and enzymatic assay.

A. Determination of Total phenols :

The total phenolic content of methanolic extracts of moth (raw and sprouts) was estimated by using Folin- Ciocalteu method and the absorbance was read at 750 nm. [12] and modified by [11].Gallic acid solution of different concentrations treated same as the samples and results were expressed as mg of Gallic acid equivalent (GAE) per 100 g of Sample (mg GAE/100g)

B. Flavonoid:

The Flavonoid content of all extracts was analysed using calorimetric method and the absorbance was read at 510 nm [13]. Rutin was taken as standard and comparison was done. The results were expressed as rutin equivalent (mg RE) / 100 g. of dry samples.

C. Determination of Total Antioxidant Capacity:

Ferric Reducing Antioxidant PowerAssay (FRAP):

FRAP assay was carried out by method developed by Benze and strain [14] and modified by et.al [11]. Trolox standard curve was developed using different aliquots and results were expressed in mg Trolox Equivalent Antioxidant capacity (TEAC) / 100g of moth.

DPPH Radical Scavenging Activity Assay (DPPH - RSA):

The method described by Bran–Williams et. al. [15] and modified by [11] Was followed for determining the antioxidant activity of the methanolic extract of moth, on the basis of the scavenging activity of the stable 2,2- Diphenyl – 1 picrylhydrazyl (DPPH) free radicals. Extract was added to methanolic solution of DPPH and absorbance at 517 nm was recorded after 20 minutes. Percentage inhibitions were calculated using the formula (1):

(A Control- A Sample/ A control)*100 and the results were expressed as mg TEAC /100 g of Moth

ABTS Radical Scavenging Activity Assay (ABTS – RSA):

The free radical – scavenging activity was determined by ABTS (2,2-Azino-bis (3–ethyl benzo thiazoline – 6- sulfonic acid diammonium salt) radical cation decolourization assay

described by RE. et. al. [15 and modified by 11]. The results were calculated using the formula (1) and the results were expressed as % of TEAC of dry weight basis.

D. Proline Assay:

Proline content was determined according to the modified method of Bates.et.al.[16].The concentration of Proline was calculated from a Proline standard curve and expressed as micromole per gram of dry sample.

E. Alpha Amylase Inhibition capacity:

α- amylase inhibition assay was determined by the slightly modified starch- iodine colour change method described by Kotowaroo et. al. 2006, Mahomoodallyet. al, 2012. [18]and modified by Anuet. al.[19]. Here 300 µl of α-amylase solution from porcine origin was added to 1 ml of soluble starch solution and 100 µl of sodium acetate buffer (0.1 M, P^H 7.2). The reaction mixture was incubated for 37 ˚Cfor 1 hr. Then, 100 µl from the reaction mixture was discharged into 3 ml of distilled water and 100 µl of iodine solution. The absorbance of the starch – iodine solution was measured at 565 nm. For assessing the potential inhibitory activity of graded concentrations of elements of moth extracts. 100 to 400 µl extract was pre incubated with 300 µl enzyme solution at 37 ˚C for 30 min. Acarbose solution was used as a positive control. Along with raw and sprouts samples similar treatment given to only starch, only Enzyme and Substrate (i.e. enzyme and starch). The results were expressed as % inhibition of enzyme activity and calculated with the following equation:

% inhibition of α– amylase activity+ $(A_{ex}- A_e)/(A_s- A_e)$ *100, where A_{ex} is the absorbance of extract, A_e is the absorbance of the enzyme and$A_s$is the absorbance of the starch.

F. Alpha – Glucosidase Inhibition capacity:

The yeast α– glucosidase was dissolved in 100 mM phosphate buffer - pH 6.8 and was used as an enzyme extract. Moth extracts were used in the concentration ranging from 75 – 150 µl. Different concentrations of moth extracts were mixed with 0.1 M phosphate buffer pH 6.8 at 37 °C for 10 minutes. 5 µl. of P-Nitrophenyl-α-D-glucopyranoside (pNPG) was used as the substrate and incubated for 75 minutes at 37 °C. It hydrolysed by α - glycosidase and release p-nitro phenol. To stop the reaction 0.1 M Sodium carbonate(0.4 ml) was added to the mixture and the colour intensity measure at 405 nm [19, 20]. The control samples were prepared without moth extracts. The glucosidase inhibitory activity was expressed as percentage inhibition.
The % inhibition was calculated according to the formula: [2]

Inhibition % $=(A_{control-} A_{sample}) A_{control} \times 100$

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) was conducted, and the difference was determined by Duncan's post hoc test considering ($P \leq 0.05$)as the significant level of difference. Pearson's coefficients (r) were also calculated to establish the relationship among various parameters. SPSS version 20 was use to carry out statistical calculations.

RESULTS AND DISCUSSION

In this study, the effect of varying concentration of ascorbic acid elicitation on antioxidants and α-amylase and α- glucosidase inhibition of Moth beans was studied.The ascorbic acid elicitor is a powerful antioxidant and is synthesized in plant cell. It is also giving good response against both biotic and abiotic stress.
The total phenolic (TP) content was 22.16 mg% in raw moth beans and was increased significantly ($p \leq 0.05$) when moth beans were germinated with(T-3 to T-6) or without (T-2) ascorbic acid elicitation (table-1). The increase in TP in control germinated moth beans (T-2) was approximately 4 times and ranged from 4.5 to 6 times in T-3 to T-6. Varying concentration of ascorbic acid had a positive and linear effect on the TP in moth beans germinated with ascorbic acid elicitation. From there results, it can be said that ascorbic acid does induced a stress like stimuli, which increase TP in moth beans. The phenolic content in germinated lentils was increase significantly in response of ascorbic acid [21]. Previous studies [8, 21,23] suggested that ascorbic acid stimulate the PPP in

legumeas it related to deNOVOsynthesis of phenolic compounds and suggested that ascorbic acid stimulate the PPP and phenyl propanol pathways in legumes.Our findings are in accordance to previous studies of sprouted peas, favabeans and kidney beans [8, 21, 23]

Phenols and flavonoidsare an excellent antioxidant and prevent the oxidative disease [24].Flavonoids protects against oxidative stress by scavenging free radical or chelating process. Some findings coincides that during germination some secondary plant metabolites such as anthocyanin and flavonoids might be produce due to biochemical metabolism [8].

The Flavonoid present in moth beans is calculated by using a standard curve prepared with Rutin. Significantly higher ($p \leq 0.05$) flavonoid content was found in moth beans germinated with ascorbic acid i.e. T-6 and lowest value was obtained in raw i.e. 56.49 mg%. The results showed the positive and linear effect on flavonoid content in moth beans germinated with ascorbic acid. Kwan Kim [25] reported that the amount of flavonoid found in the mungsprouts lower than the moth beans compared to the raw moth beans.

Proline is found in higher amount in plant cell in response of various stress to plant. It is synthesized from glutamate [26]. This higher amount of proline in cell is basically due to deNOVO synthesis [27]. Proline can also provide stability to cellular components and act free radical scavenger [28]. Studies [26, 24] reported that stimulation of PPP increase proline content. The proline content was increased significantly ($P \leq 0.05$) in T-2 and T-3 to T-6 compared to raw moth beans (T-1). The ascorbic acid did not show the elicitation effect on Proline. In this study germination had a significant impact on Proline.

The total antioxidant capacity (TAC) is a total response of antioxidant compound to neutralize the free radicals in a system. There are many *In vitro* methods developed to measure TAC in plant foods with different principles. In this study FRAP, DPPH-RSA and ABTS-RSA were used to measure TAC of control and germinated moth beans. As many studies reported a positive and significant correlation among this three different *in vitro* total antioxidant capacity method.[8,22]

FRAP assay measures the antioxidant effect of substance in the reaction medium and it shows the total antioxidant power. The process is based on the ability of the samples to reduce $Fe^{3+}$to Fe^{2+} ions. The reducing power of the germinated samples was found higher than the raw. As germinated samples contain higher antioxidants which causes a higher reducing power [23].The FRAP was increased in germinated moth beans (T-2) and germinated moth beans with elicitation (T-3 to T-6) compared to T–1. The elicitation of moth beans with ascorbic acid showed a significant effect on FRAP. The FRAP content was increased approximately 8.5 to 9 times in T-2 to T-6 than the raw moth beans.

DPPH (2,2- Diphenyl – 1- picrylhydrazyl), is a stable organic free radical and but the absorption disappear due to reduction by an antioxidant. Formation of non –radical form (DPPH- H) is occurs due to the presence of hydrogen donating antioxidant compound and reduction of methanolic DPPH solution. Maximum adsorption of DPPH occurs at 517nm by accepting an electron[22].The methanolic extract of moth beans could contain some substances which are electron donors which convert free radicals to more stable product and stop the radical chain reactions.

ABTS radical cation was produced in the stable form using potassium persulphate and has been compared with standard Trolox. The moth beans extract was added to the reaction medium and the antioxidant power was measured by studying decolourization.Raw and germinated moth beans without ascorbic acid elicitation showed the similar ABTS–RSA. The T-3 to T–6 showed a significantly higher ABTS- RSA compared to T-1. There was a significant increase in ABTS-RSA when moth beans were germinated with ascorbic acid elicitation. There was no any linear response of ascorbic acid concentration on ABTS-RSA.

The results of FRAP, DPPH-RSA and ABTS-RSA are graphically presented in Figure.1. The increased total antioxidant in response of ascorbic acid elicitation is probably due to stimulation of PPP in legume [23].As mentioned earlier ascorbic acid is a potential antioxidant which might be a second possible cause of increasedTAC in germinated moth beans. Antioxidant activity was increased in dark

germinated favabeans sprouts in response of ascorbic acid eliciation and correlated the increased TAC with increased SOD and catalase activity. Various studies [19, 23, 24]reported increase in TAC in lentils, Fava beans, African yam beans and other legumes. Randhir et. al.[24] reported that the antioxidant activity was high in fava beans elicited with ascorbic acid due to increase in L – DOPA and phenolics content which might be contribute to increase the TAC.

Recently, dietary management has gain importance to control blood glucose levelabove 180 mg / dlin diabetics is considered as risk for developing secondary complications. The food rich in α- amylase and α- glucosidase inhibitors are known as hypoglycemic foods. They both are key enzymes to manage hyper glycemia[27].α- amylase inhibitors are starch blockers, which can bind with the reactive sites of amylase enzyme and reduce blood sugar level by altering its catalytic activity.The inhibitory effect of α-amylase and α- glucosidase in moth beans sprouts are graphically presented in Figure.2

The extract of moth beans (T-6) showed highest inhibitory activity Whereas T-1 showed 12.41 % of inhibition. There was no effect of ascorbic acid elicitation on α-amylase inhibition in moth beans.There results revealed that germination had a good impact on α- amylase inhibition and methanolic extract showed a comparable results with acarbose.

The α-glucosidase inhibition in raw moth beans was 95.2 %. Germination with (T-3 to T-6) and without (T-2) ascorbic acid elicitation did not showed any significant effect on α- glucosidase inhibition (table-2). The presence of phenolics and Flavonoids in the germinated legumes showed inhibitory effect on α- amylase and α-glucosidase activity.

In this study Pearson's correlation was studied to evaluate a relationship between various biochemical parameters. The results obtained are shown in table 2. The total phenolics had a positive and a significant correlation with Flavonoid, Proline Total antioxidant capacity and alpha amylase inhibition. It had a negative and significant correlation with alpha glucosidase inhibition. The proline had a similar trend of relation with Total phenol, Flavonoid, Alpha amylase, Total anti-oxidant capacity and alpha glucosidase. The correlation between three different methods of Total anti-oxidant capacity was found to be positive and significant.

CONCLUSION:

From the results of this study, the application of moth bean seed elicitation with ascorbic acid had a significant advantage to improve phenolic bioactive compounds which have linked with total antioxidant capacity and hypoglycaemic function. Therefore moth bean sprouts can be used in daily diet or for formulating food for diabetic populations.

REFERENCES:

[1]. Kahn, S. E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*, *46*(1), 3-19.

[2]. Nair, S. S., Kavrekar, V., & Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology*, *3*(1), 128-132.

[3]. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR (2015) Management of hyperglycaemia in T2- D, 2015: a patient-centred approach. Update to a position statement of the American diabetes association and the European association for the study of diabetes. Diabetologia. *Diabetes Care*, 38, 140–149 DOI: 10.2337/dc14-2441

[4]. Ali Asgar, M. D. (2013). Anti-diabetic potential of phenolic compounds: A review. *International Journal of Food Properties*, *16*(1), 91-103.

[5]. Tundis, R., Loizzo, M. R., & Menichini, F. (2010). Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini reviews in medicinal chemistry*, *10*(4), 315-331.

[6]. Baenas, N., García-Viguera, C., & Moreno, D. (2014). Elicitation: a tool for enriching the bioactive composition of foods. *Molecules*, *19*(9), 13541-13563.

[7]. Andarwulan, N., & Shetty, K. (1999). Improvement of pea (Pisum sativum) seed vigour response by fish protein hydrolysates in combination with acetyl salicylic acid. *Process Biochemistry*, *35*(1-2), 159-165.

[8]. Randhir, R., Lin, Y. T., & Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*, *39*(5), 637-646.

[9]. Randhir, R., Kwon, Y. I., & Shetty, K. (2009). Improved health-relevant functionality in dark germinated Mucuna pruriens sprouts by elicitation with peptide and phytochemical elicitors. *Bioresource technology*, *100*(19), 4507-4514.

[10]. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology* , 299, 152-178.

[11]. Parikh, B., & Patel, V. H. (2018). Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses. *Journal of food science and technology*, *55*(4), 1499-1507.

[12]. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.

[13]. Zhishen Jia, Mengcheng Tang, Jianming Wu. (1999): The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Journal of food Chemistry, 64: 555-559.

[14]. Brand-Williams, W., Cuvelier, M. E., &Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

[15]. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cationdecolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

[16]. Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

[17]. Kotowaroo, M. I., Mahomoodally, M. F., Gurib-Fakim, A., & Subratty, A. H. (2006). Screening of traditional antidiabetic medicinal plants of mauritius for possible α-amylase inhibitory effects in vitro. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *20*(3), 228-231.

[18]. Mishra, A., Patel, V.H.,(2017).Evaluation of alpha amylase, alpha glucosidase and protein glycation inhibitory activities of different elements of mango (Mangiferaindica), *PRAJNA- Journal of pure and applied science*, 24-25: 174-181.

[19]. Wang, T., Li, X., Zhou, B., Li, H., Zeng, J., & Gao, W. (2015). Anti-diabetic activity in type 2 diabetic mice and α-glucosidase inhibitory, antioxidant and anti-inflammatory potential of chemically profiled pear peel and pulp extracts (Pyrus spp.). *Journal of Functional foods*, *13*, 276-288.

[20]. Peñas, E., Limón, R. I., Martínez-Villaluenga, C., Restani, P., Pihlanto, A., & Frias, J. (2015). Impact of elicitation on antioxidant and potential antihypertensive properties of lentil sprouts. *Plant foods for human nutrition*, *70*(4), 401-407.

[21]. Uchegbu, N. N., & Amulu, F. N. (2015). Effect of Germination on Proximate, Available Phenol and Flavonoid Content, and Antioxidant Activities of African Yam Bean (Sphenostylis stenocarpa). *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*, *9*(1), 106-9.

[22]. Burguieres, E., McCue, P., Kwon, Y. I., & Shetty, K. (2007). Effect of vitamin C and folic acid on seed vigour response and phenolic-linked antioxidant activity. *Bioresource Technology*, *98*(7), 1393-1404.

[23]. Randhir, R., & Shetty, K. (2007). Elicitation of the proline-linked pentose phosphate pathway metabolites and antioxidant enzyme response by ascorbic acid in dark germinated fava bean sprouts. *Journal of food biochemistry*, *31*(4), 485-508.

[24]. Kwak, S. S., Kim, S. K., Park, I. H., & Liu, J. R. (1996). Enhancement of peroxidase activity by stress-related chemicals in sweet potato. *Phytochemistry*, *43*(3), 565-568.

[25]. Mehta, S. K., & Gaur, J. P. (1999). Heavy-metal-induced proline accumulation and its role in ameliorating metal toxicity in Chlorella vulgaris. *The New Phytologist*, *143*(2), 253-259.

[26]. Peng, Z., Lu, Q., & Verma, D. P. S. (1996). Reciprocal regulation of Δ 1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. *Molecular and General Genetics MGG*, *253*(3), 334-341.

[27]. Kwon, Y. I. I., Vattem, D. A., & Shetty, K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia pacific journal of clinical nutrition*, *15*(1), 107-118.

Table: 1 The total phenolics, Flavonoid and a Proline content of the raw, and germinated moth beans with ascorbic acid elicitor

Treatments	Total Phenols	Flavonoids	Proline
T-1	22.16 ± 0.98[a]	56.49 ± 4.00[a]	20.0 ± 0.44[a]
T-2	84.09± 1.94[b]	116.18 ± 1.60[b]	117.02 ± 1.05[d]
T-3	102.9 3 ± 5.02[c]	119.79 ± 8.00[b]	121.30 ± 6.99 [d]
T-4	114.7 ± 5.38[d]	135.24 ± 2.90[b]	85.57 ± 1.45 [b]
T-5	120.4 ± 4.25[d]	144.23 ± 2.92 [d]	97.03 ± 2.11 [c]
T-6	135.3 ± 7.34[e]	158.64 ± 4.12[e]	115.24 ± 3.06[d]
F- Value	297.91	260.93	526.81

Values are mean of ± S.D. of four observations. Mean value of different superscripts within a column are significantly different from each other ($p \leq 0.05$). GAE-Gallic acid equivalent, TE- Trolox – equivalent.

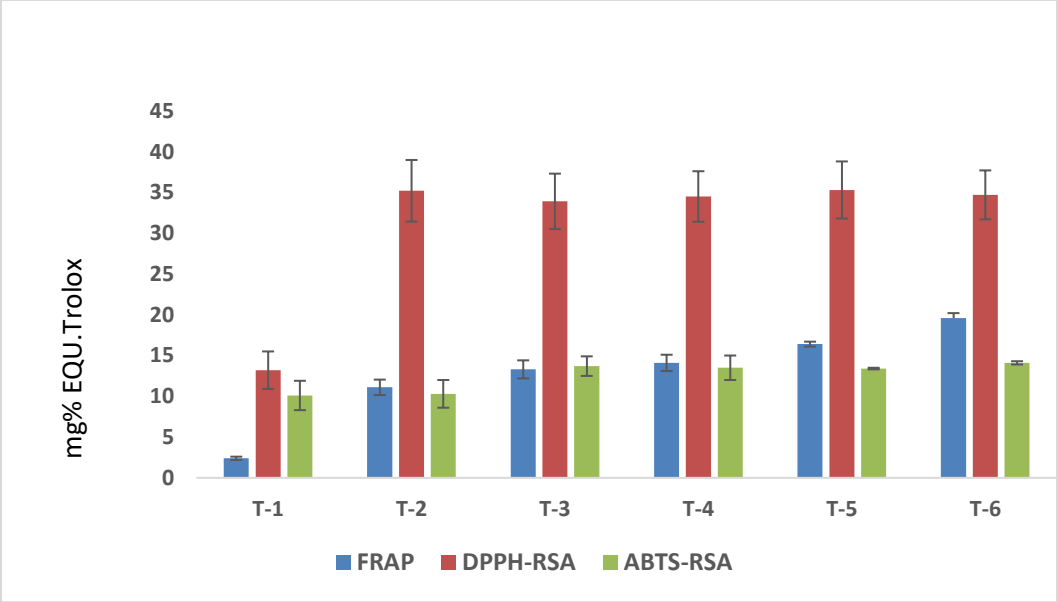

Fig.1 FRAP, DPPH –RSAand ABTS -RSA content of the Raw and germinated Mothbeans

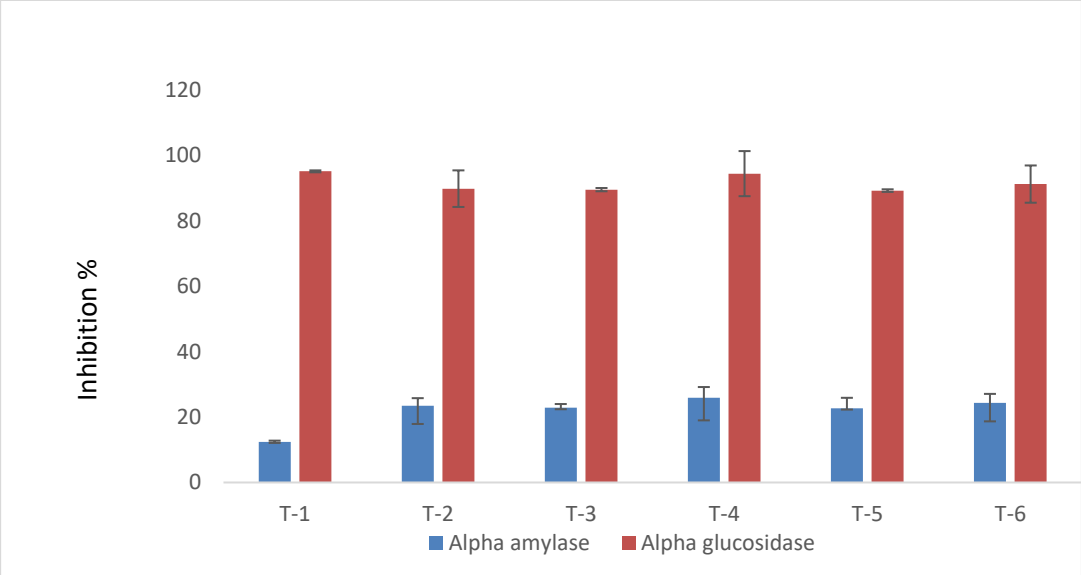

Figure: 2 Inhibitory effects of α- amylase and α- glucosidase in moth beans sprouts

Table:2 Pearson correlation among various parameters

	Total Phenolic	Flavonoids	FRAP	DPPH-RSA	ABTS-RSA	Proline	α-amylase inhibition	α- glucosidase inhibition
Total Phenolic	1	.981**	.969**	.873**	.687**	.785**	.772	.239**
Flavonoids	.981**	1	.982**	.845**	.651**	.787**	.796**	-.264
FRAP	.969**	.982**	1	.786**	.717**	.790**	.794**	-.310**
DPPH-RSA	.873**	.845**	.786**	1	.368*	.861**	.695**	-.197**
ABTS-RSA	.687**	.651**	.717**	.368*	1	.468*	583**	-.317**
Proline	.785**	.787**	.790**	.861*	.468*	1	.776**	-.423**
α- amylase inhibition	.772**	.796**	.794**	.695*	.583**	.776**	1	-.432**
α-glucosidase inhibition	-.239	-.264	-.310	-.197	-.317	-.423*	-.432	1

**correlation significant at the 0.01 level (1- tailed)
*Correlation is significant at the 0.05 level (1- tailed)