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Effect of bio-fertilizer and Inorganic fertilizers on enzymes activity of *Arachis hypogaea* L

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Abstract
The current investigation was made to analyze the impact of different treatments on *Arbuscular Mycorrhizal* Fungi (AMF), *Azospirillum* (AZM), Market waste compost (MWC), Nitrogen (N), Phosphorus (P) and Potassium (K) are organic and inorganic fertilizers on several antioxidant enzyme activities like Catalase (CAT), Peroxidase (POD) and Polyphenoloxidase (PPO) of *Arachis hypogaea* L. To analysis of the enzyme activities were estimated at different day's intervals. The results of this experiment revealed that combined of bio-fertilizers (T4) significantly increased antioxidant enzyme activities of the crop plant. In general, it might be concluded, further study on the log term of application of bio-fertilizers should be good tool to improve the crop productivity and mass and soil health.

Keywords: AMF, enzymes, fertilizers, groundnut

Introduction
Groundnut is an essential grain legume produces in worldwide. India is the third biggest manufacturer of oil seeds in the world. It statement for 19% of area and 9% of the global production [12]. One of the most significant roles of agriculture crop production is to provide about every essential minerals and organic nutrients to humans. Proteins and vitamins of essential human nutrients are present known to be of great significance in maintaining human health. The bio-fertilizers besides providing a good substrate for crop growth facilitate to propagate advantageous microbes in soil and in addition provide residual effect of subsequent crops.
The growth stimulating bacteria *Azospirillum* which, moreover to biological fixation of nitrogen and solubilization of soil [2]. *Arbuscular mycorrhizal* fungi (AMF) are among the most general soil fungi and the mass of plant genus have associations with AMF species. Consequently, it becomes indispensable to survey the most effective mixture of bio-fertilizers and inorganic fertilizers for sustaining the soil fertility and producing class productions. Inorganic fertilizers is believed to shrink the antioxidant levels whereas, organic fertilizers has been demonstrated to enhance antioxidant in plants [5]. Applying, fertilizers predominantly in the inorganic form, in excess of plant requirements can enlarge the chances of fertilizers loss and environmental pollution. Organic fertilizers, apart from civilizing physical and biological properties of soil, aid in improving the efficiency of chemical fertilizers. In the present study, was conducted to estimate the effects of application of bio-fertilizers and inorganic fertilizer (NPK) on enzymes activities responses of groundnut.

Materials and Methods
Experimental site
The study was conducted as field experimental at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, India.

Cultivar
The seeds of groundnut (*Arachis hypogaea* L.) var. VRI 2 were obtained from Regional Research Station of Tamil Nadu Agricultural University, Virudhachalam, Cuddalore District, Tamil Nadu, India.

Methods
The experiment was conducted in randomized block design with five replications treatments. The treatments were T1- Control, T2- *Arbuscular Mycorrhizal* Fungi (AMF), T3- *Azospirillum* (AZM), T4- Market waste compost (MWC), T5- AMF+AZM+ MWC, T6- Nitrogen (N), T7- Phosphorus (P), T8- Potassium (K) and T9- N+P+K. The garden soil, selected groundnut seeds were sown in the field irrigated with normal tap water was maintained as the control. Various growth and biochemical parameters were recorded after 15, 30, 45, 60 and 90th days.

Fertilizers
The AM Fungi (*Glomus fasciculatum*) and *Azospirillum* were collected from Department of Microbiology Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. Raw organic material vegetables wastage enhance their suitability for application to the soil as a fertilizer resource, after having undergone composting.

Enzymes studies
The following methods were used for estimation of Catalase (Machly and Chance, 1967), Peroxidase and Polyphenoloxidase (Kumar and Khan, 1982).

Results and Discussions
Modify in the activity of antioxidant enzymes in a defense mechanism of plants underneath oxidative stress induced environmental stresses [6]. The amount of catalase (CAT) recorded in control group was 2.16 (moles of H_2O_2 reduction/mg protein) at 15th days of experiment and the value were increased upto 6.74 (moles of H_2O_2 reduction/mg protein) at 60th days. The amount of CAT

showed a significant increase in both fertilizers treatments when compared to the control plants (Table. 1). Among the organic fertilizers treatment, combined treatments reported the highest seedlings value was (T4) 9.84 (moles of H_2O_2 reduction/mg protein) at 60th days of experiment. While, inorganic fertilizer treatments, the NPK treatment reported the highest quantity of CAT which value was (T9) 7.56 (moles of H_2O_2 reduction/mg protein) at 60th days. Antioxidative enzymes like catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) are most significant components in the scavenging system of reactive oxygen species (ROS) [10]. The increase in CAT activity by the relevance of bio-fertilizers in our study may have accelerated the metabolic process of aerobic organism. Earlier, studies have shown that the processes play an important role in controlling the liberate of biologically presented nutrients from organic components [13]. The increase in CAT activity may designate that the combination of filtered mud and plant growth promoting rhizobacteria resulted hydrogen peroxide (H_2O_2) synthesis, thus inducing the production of different oxidative defense enzymes [4].The amount of peroxidase (POD) in control groundnut leaf is 0.89 (µmol/min./mg protein) at 15th days. The values were increased as 30th, 45th, 60th, and 90th days of experiment. Among the organic compost treatments, combined treatments reported the highest peroxidase content was (T4) 6.82 (µmol/min./mg protein) at 60th days (Table.2). While, inorganic fertilizer treatments, reported the highest quantity of peroxidase activity was (T9) 4.09 (µmol/min./mg protein) at 60th days of experiment. Among the treatments, organic fertilizers reported significant increase in peroxidase activity when compared to inorganic fertilizers and control plants. Peroxidase enzymes showed increased activity with different organic fertilizers, similar results were reported by [1] in sunflower with nitrogen and phosphorus fertilizer and confirmed by [8]. According to them, these responses can be recognized as an attempt of the plant to defeat the adverse conditions of several elements necessary for growth and development of groundnut plants.

The effect of organic and inorganic fertilizers treatments on polyphenoloxidase activity of leaf of *Arachis hypogaea* at different stages of interval is represented in Table.3. Both the fertilizers treatments had significant effect on polyphenoloxidase (PPO) activity when compared to the control. Among the organic fertilizer treatments, the highest PPO activity was observed at combined treatment (T4) followed T1, T2 and T3 at 60th days. In both fertilizers, combined treatments reported the highest reading, even through organic compost treatment is found to be more efficient in inducing PPO activity. The decrease in polyphenoloxidase activity may also be explained by decreased phosphorus [3]. Phosphorus is narrowly connected to energy transfer process in cells, particularly the synthesis of adenosine triphosphate (ATP) and amino acids, therefore of affecting polyphenoloxidase [11].

Plant product themselves from cytotoxic effects of aforementioned reactive oxygen species (ROS) with the help of antioxidant enzymes such as catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) are induced in plants in reaction to the stress [7]. Induced oxidative response enables the plants to cope with different kinds stresses and allows them to be phenotypically plastic. It makes the plant additional changeable for stress causing agents due to the variations in defense constituents of the plants [14, 15]. Nitrate reductase activity in plants, which donations to plant nitrate assimilation is influenced not only by ecological factors such as light, humidity and temperature, but also by soil nutrient concentrations including nitrogen, phosphorus and potassium [9]. According to them, enlarge in enzyme activities could be due to interaction of available rhizobacteria and micorrhizal fungi with roots of bio-fertilizers and inorganic fertilizers.

Table 1: Effect of organic and inorganic fertilizers on catalase of groundnut

Treatments	Catalase (moles of H_2O_2 reduction/mg protein) Treatment days				
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS
T1	2.16	4.04	6.18	6.74	5.81
T2	3.19	5.92	7.32	7.92	6.82
T3	3.42	6.12	7.84	8.48	6.99
T4	3.84	6.42	7.98	8.62	7.14
T5	4.23	7.42	9.23	9.84	8.19
T6	2.20	4.15	6.24	6.82	5.91
T7	2.64	4.49	6.52	6.98	6.24
T8	2.89	4.62	6.72	7.10	6.34
T9	3.03	5.17	7.10	7.56	6.72

Table 2

Anova Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	33.54024	8	4.192531	73.32982	2.19E-18	2.244396
Columns	127.5878	4	31.89696	557.8966	4.27E-29	2.668437
Error	1.829556	32	0.057174			
Total 162.9576		44				

Table 3: Effect of organic and inorganic fertilizers on peroxidase of groundnut

Treatments	Peroxidase (µmol/min./mg protein) Treatment days				
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS
T1	0.89	1.52	2.10	2.89	1.94
T2	1.92	3.14	4.42	4.74	4.12
T3	2.41	3.89	4.62	5.12	4.29
T4	2.81	4.10	5.14	5.92	4.62
T5	3.52	5.12	6.24	6.82	5.19
T6	1.14	1.62	2.32	3.01	2.10
T7	1.32	1.84	2.82	3.39	2.52
T8	1.52	2.18	3.14	3.38	2.89
T9	1.89	3.24	4.10	4.09	3.14

Table 4

Anova Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	57.45598	8	7.181997	91.04501	8.31E-20	2.244396
Columns	33.06371	4	8.265928	104.7858	6.51E-18	2.668437
Error	2.524289	32	0.078884			
Total	93.04398	44				

Table 5: Effect of organic and inorganic fertilizers on polyphenoloxidase of groundnut

Treatments	Polyphenoloxidase (µmol/min./mg protein)				
	Treatment days				
	15 DAS	30 DAS	45 DAS	60 DAS	90 DAS
T1	1.57	2.36	3.45	4.10	3.58
T2	2.14	3.17	3.92	5.28	4.29
T3	2.32	3.34	4.19	5.62	4.41
T4	2.62	3.62	4.31	5.81	4.62
T5	3.12	4.28	5.20	6.41	5.14
T6	1.62	2.41	3.51	4.28	3.62
T7	1.84	2.59	3.63	4.34	3.79
T8	1.91	2.71	3.42	4.48	3.84
T9	2.10	3.04	3.80	5.17	4.10

Table 6

ANOVA						
Source of Variation	**SS**	**Df**	**MS**	**F**	**P-value**	**F crit**
Rows	14.20742	8	1.775927	73.96444	1.93E-18	2.244396
Columns	44.5049	4	11.12623	463.3889	7.92E-28	2.668437
Error	0.768338	32	0.024011			
Total	59.48066	44				

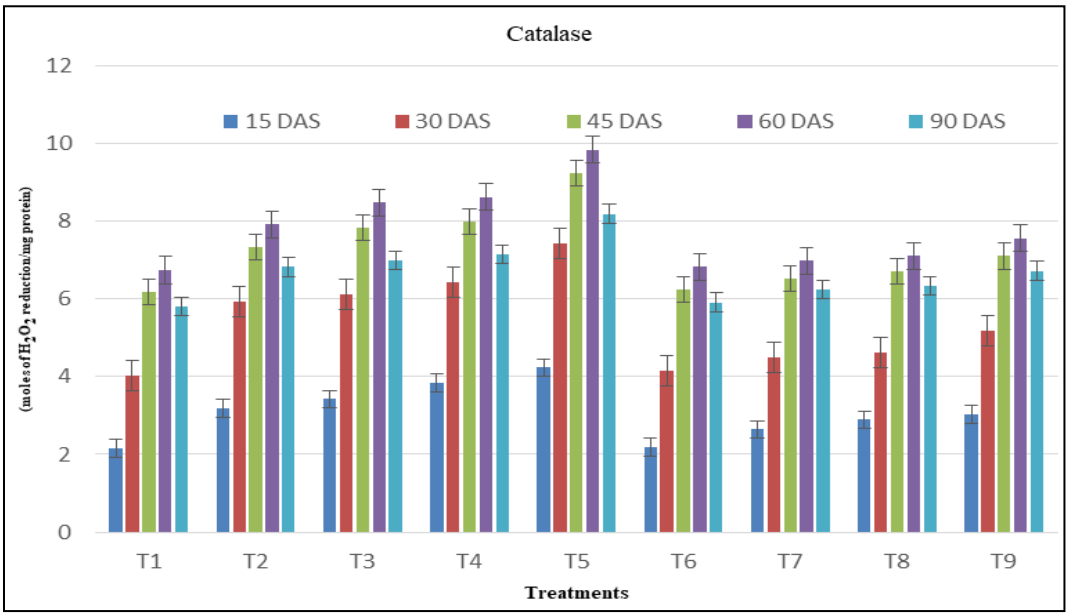

Fig 1

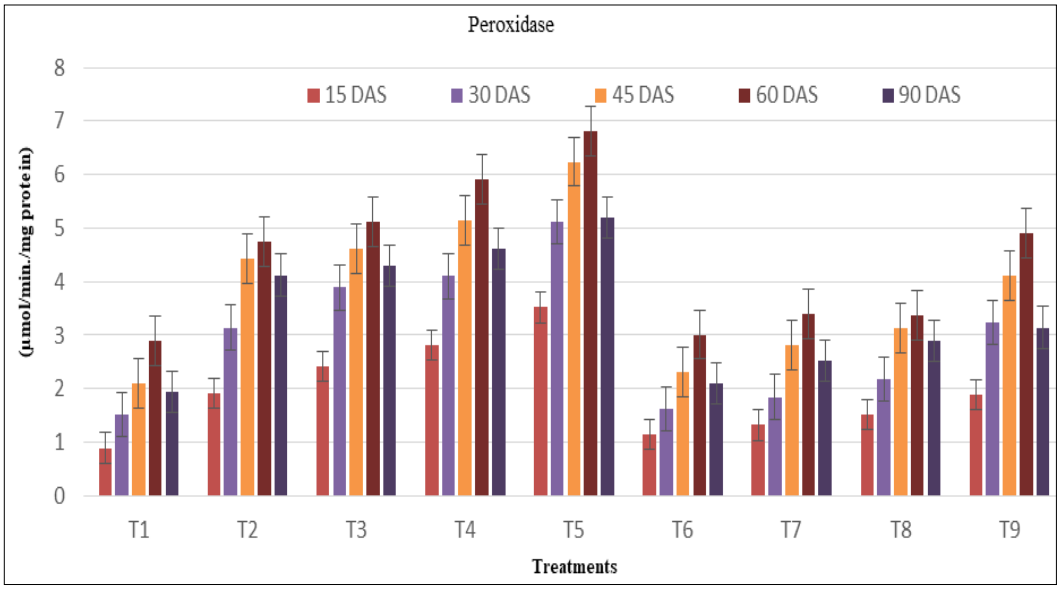

Fig 2

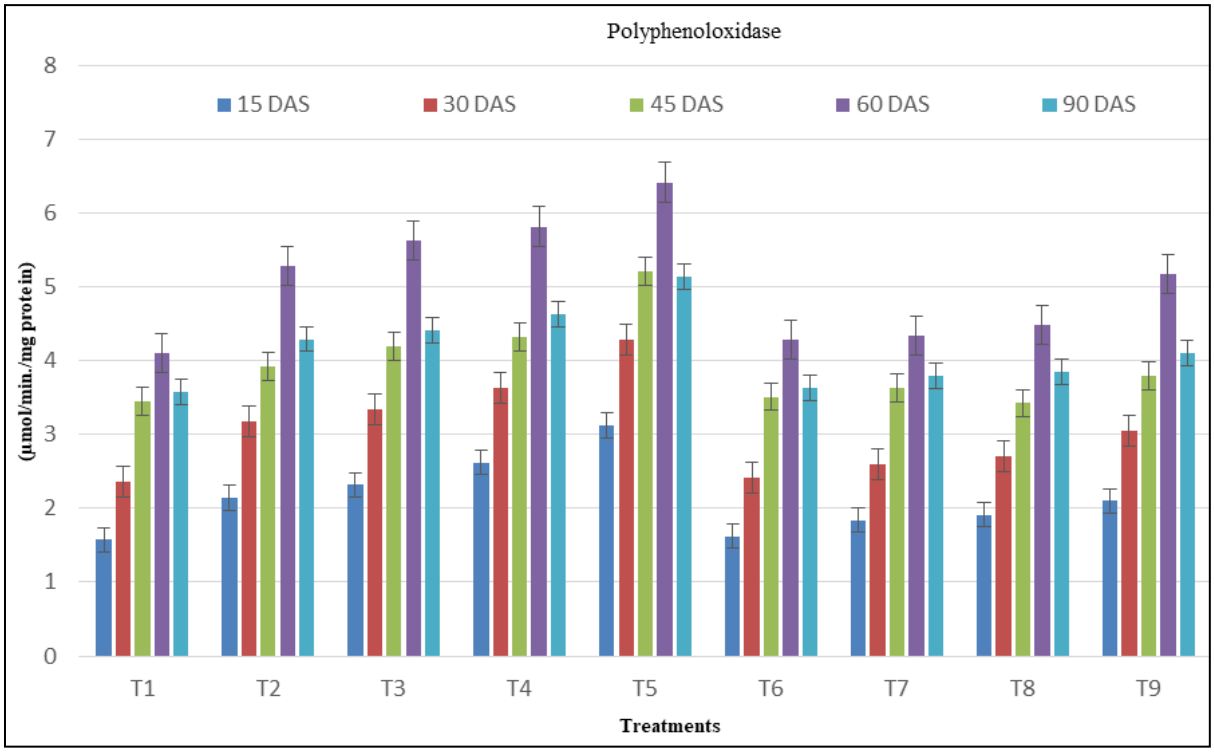

Fig 3

Conclusions

In the study, indicates that the groundnut showed greater response to the application of organic fertilizers treatments. The enzyme activities such as catalase (CAT), peroxidase (POD) and polyphenoloxidase (PPO) are higher in groundnut plant growth with combined application of bio-fertilizers higher than inorganic fertilizers. The residual toxicities of inorganic fertilizers deception problem of environment pollution, reduction of essential nutrients due to random use of inorganic fertilizers which has hazard to the sustainability of crop invention. The bio-fertilizers besides providing a good substrate for crop growth facilitate to propagate beneficial microbes in soil.

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