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Effect of Cadmium on Total Starch and Lipid Content of *Pisum Sativum* Treated With Mycorrhiza and Putrescine

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Running Head: Mycorrhiza and Putrescine guard Pisum Sativum grown in Cd-toxicity

Keywords: Cadmium toxicity (Cd), Pea, Starch, Lipid, Mycorrhiza, and Putrescine

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Abstract

Cadmium (Cd), Mercury (Hg), and Arsenic (As) which are few toxic heavy metals play a havoc health condition for the mass. However, the response of our food harvesting crops on these intoxicating heavy metals is a matter of great concern as we heavily rely on those as food resources. Our current study objectified on *Pisum sativum*, a pea variety. We designed this research work for understanding the protective as well as the preventive role of polyamines like Putrescine as well as mycorrhiza for *Pisum sativum* under the exogenously generated stress by the addition of cadmium nitrate in the soil. In this study, we estimated the total level of starch and lipid content in the presence and absence of putrescine and mycorrhiza when the plant was grown at Cd-toxicity. The results obtained from this study are quite noteworthy for learning the protective and preventive nature of putrescine and

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mycorrhiza for improving the damaging effect of heavy metal toxicity and other adverse circumstances.

Introduction

Cadmium toxicity induced oxidative stresses in the plants. Cadmium is bioavailable and can be easily transported inside plants as a metallo-organic complex [1]. Polyamines are low molecular weight aliphatic polycations which can readily bind with the ROS anionic species. They act as a protective shield to protect membranes and biomolecules by binding with the negatively charges surface [2]. This increase of free radicals like superoxide, hydroxyl radical, hydrogen peroxide, etc., interfered with the metabolic activity and disrupted cell structure, membrane stability, and integrity. It reduced the chlorophyll content of Chl a, Chl b, Chl c, [3]. The toxic effect of cadmium caused chlorosis, necrosis, marginal yellowing in *Pisum sativum* (Pandey and Singh 2012). Oxidative stress increased the production of MDA and H_2O_2 drastically [4]. According to the report of [5], the photosynthetic activity was affected due to decreased chlorophyll content.

For this research work, we chose a pea plant, because, research on the fatal impact of Cd-toxicity on pea plants is less explored. Secondly, the level of sensitivity of pea which is often as protein feed sources and quality assurance particular in the unease with cadmium for heavy metal is still unknown; especially on the level of total starch and lipid content. The objectives here were to study a. the effect of cadmium stress on total starch and Lipid of a pea, and b. the effect of polyamines (especially putrescine) and mycorrhiza in neutralizing the damaging effect of Cd-toxicity.

Materials and methods

The pot experiment was performed with one genotype of pea Arkel in the polyhouse of the School of Agriculture, Lovely Professional University, Jalandhar, Punjab. Pea genotype from the University of Agriculture in Punjab, Punjab. The pot size for the experiment was 30 cm in diameter and 25 cm in height with 14 kg of soil capacity each. Pisum sativum seeds were seeded in each pot. Targeted pots have been inoculated with Endomycorrhiza Glomus sp, according to the work plan. After that, the exogenous application of cadmium nitrate in the soil created heavy metal stress in the plant. One best concentration within the range of 1-100 ppm of selected cadmium after the initial screening was 100 ppm. To create stress in pea plants, this selected concentration of heavy metals was applied in the soil. Putrescine was applied in a foliar application at an interval of 15 days at a

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rate of 5ppm and 10ppm. The specific observations were made after sowing at three points such as 30, 60 and 90 DAS. In a completely randomized design (CRD), the experiment was laid out. Ten therapies, including command, are available. Three times each treatment has been replicated.

Total Starch (mg g^{-1} FW)

The total starch content of the plant sample was estimated using Sadasuvam and Manickam's proposed method [6]. To remove sugars, the sample is treated with 80% alcohol and then starch is extracted with perchloric acid. Starch is hydrolyzed to glucose in a hot acid medium and dehydrated to furfural hydroxymethyl. This compound forms an anthrone-colored green product. The 500 mg leaf test was homogenized to extract the sugars in warm 80% ethanol. The sample was 20 min at 5000 rpm centrifuged. This removed the supernatant and kept the remainder. This residue was repeatedly washed with 80% hot ethanol until anthrone reagent does not give color to the washing. The specimen was well dried in a bath of water. The residue was added to 5.0 ml of water and 6.5 ml of 52 percent perchloric acid. Using cooling centrifuge, the mixture was centrifuged at 0 ° C at 5000 rpm for 20 min. The collected supernatant has been preserved. Using fresh perchloric acid, the extraction was repeated. Collected the supernatant and made up to 100 ml. The pipette was 0.1 or 0.2 ml supernatant and the final volume was 1 ml. Then there was inserted 4 ml of the anthrone reagent. Then it was cooked in a boiling tub of water for 8 minutes. The solution was cooled and a spectrophotometer was used to determine the strength of green to dark green color at 630 nm. To reach the starch content in the sample, the value obtained was multiplied by a factor 0.9. The 100 mg of glucose was dissolved in 100 ml of distilled water and the working form was prepared with 100 ml of distilled water by dilution of 10 ml of normal glucose inventory. By taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in a separate test tube, different concentrations of the sugar solution were prepared from this stock solution. After incorporating distilled water, the final volume of these test tubes was rendered to 3 liters and 6 ml of the anthrone reagent was applied to each test tube. In a water bath, the solution was heated. The solution was cooled and the blue color intensity at 620 nm was read. By plotting the absorbance price on the y-axis against the sugar intensity on the x-axis, the typical curve was planned. The resulting value was multiplied by a factor of 0.9 to reach the sample's total starch content.

Estimation of Total Lipid

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Jayaraman's following method [7] estimated the maximum lipid content in the leaves. The tissue is collected in a mixture of 3:1 ether and ethanol accompanied by centrifugation and segregated by introducing the KCl solution which helps to prevent emulsification in surface isolation and salt. Then the lipid surface is washed, measured, and determined the volume of lipid. For 10 ml of solvent mixture i.e., ethyl either and ethanol, the 1.0 g of the fresh specimen was homogenized. At 2000 g for 10 min, this was centrifuged and the supernatant was then moved to a different funnel. The 2 ml solution of 0.05 M KCl was added to the extract and well shaken. There were two separate layers. One layer was a lipid, while the other layer was water. Both layers were carefully decreed. Then dried and measured the lipid surface.

Results and Discussion

Total Starch

In pea variety Arkel under cadmium strain, the effect of polyamine (putrescine), mycorrhiza and their combination on total starch (mg g-1) were observed. At 30, 60 and 90 days after sowing (DAS) data were recorded (Table 1 & Fig. 1). It is clear that, when subject to heavy metal pressure (T2) at 30, 60 and 90 DAS intervals, the average total starch decreased significantly by 63.39 percent, 63.78 percent, and 18.42 percent relative to command (T1). Exogenous application of endomycorrhiza in soil (T3) demonstrated the benefit of reduction by increasing the total starch by 59.41 percent, 75.58 percent and 30.33 percent were relative to T2 at 30, 60 and 90 DAS. Compared to T2, putrescine (T6) exogenous application showed a mitigating effect by increasing the total starch by 36.62 percent, 36.73 percent and 13.44 percent of the proposed DAS. The average total starch was significantly increased by 80.64 percent compared to T2; 81.72 percent; and 39.80 percent compared to (T2) when treated with a higher dose of putrescine (T5). Similarly, the average total starch increased significantly with 69.14 percent, 44.76 percent and 15.06 percent at the proposed DAS when treatment was compared with T7The average total starch increased significantly when administered with a higher dose of putrescine relative to T8 by 58.47 percent, 52.46 percent, and 21.11 percent compared to T2. The mixture of putrescine and mycorrhiza showed the best mitigating impact in T9 treatment by increasing total starch by 65.75%, 64.42% and 28.73% in the planned DAS therapy. Compared with T2 treatment, T10 increased significant total starch by 66.47 percent, 61.55 percent, and 30.01 percent, respectively. The average total starch in this therapy (T4) was found to increase dramatically by 69.28 percent, 81.44 percent respectively, and 37.37 percent relative to T2 in the planned DAS. In terms of enhanced total starch, the effect of putrescine at 5 ppm on 30 DAS and putrescine at 60 DAS

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and 90 DAS shows the best mitigating effect. The reported decrease in total starch content concerning high zinc levels may be attributable to its function in enzymatic reactions linked to carbohydrate catabolism cycles [8] or possibly leading to photosynthetic inhibition or breathing speed stimulation [9]. A drop was noticed in overall starch under heavy metal stress in *Albizia Procera* [10]. Both heavy metals have reduced the quality of crops with rising concentrations [11].

Total Lipid

In pea variety Arkel under cadmium pressure, the impact of polyamine (putrescine), mycorrhiza and their combination on total lipid (mg g-1 FW) are observed. It is clear that, when subject to heavy metal pressure (T2) at 30, 60 and 90 DAS levels, the average total lipid content decreased significantly by 8.88%, 41.38%, and 13.75% relative to control (T1). Exogenous application of endomycorrhiza in soil (T3) demonstrated the mitigation impact by increasing the average total lipid by 32.41%, 55.52% and 21.53% relative to T2 at 30, 60 and 90 DAS. Compared to T2, putrescine (T6) exogenous application showed a mitigating effect by increasing total lipid by 41.14%, 31.14% and 17.15% on proposed DAS. The average total lipid was significantly increased by 68.46%, 45.95%, and 27.56% when administered with a higher dose of putrescine (T5) relative with T2. Likewise, as T2 was associated with T7, the overall lipid increased significantly at the suggested DAS by 57.94 percent, 39.50 percent, and 18.51 percent. The average total lipid content increased significantly when administered with a higher dose of putrescine relative to T2 compared to lT8 by 18.81 percent, 54.62 percent, and 19.51 percent. Combining putrescine and mycorrhiza in the case of T9 was attained by increasing total lipid at the suggested DAS by 68.21 percent, 63.43 percent and 29.03 respectively. By compared T10 treatment with T2 care, substantial overall lipid improved by 62.03%, 65.37%, and 32.88%, respectively. Therefore, putrescine's effect on 30 DAS at 10 ppm, mycorrhiza at 60 & 90 DAS, putrescine (10 ppm) + mycorrhiza show the best protective effect in terms of improved total lipid content. Phytoremediation can be used in plant organs [12] to hyper accumulate metals. This method of soil-based metal improvement can be enhanced by introducing the plants to certain metal-resistant bacteria that are associated with improved plant growth and can also bind metals in plant tissues. Also proposed research on cadmium-resistant plant growth inoculation enlightened that rhizobacteria improve agricultural production that will prevent metal accumulation in plant tissues. With limited or no deposition of cadmium in the edible parts of crops, the use of microorganisms may stimulate plant growth in soils polluted with cadmium.

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Conclusion

Our fertile agricultural land is currently getting polluted by toxic heavy metals like Cd, Hg, etc coming from industrial waste; especially chemical industries. Therefore, it is necessary to concentrate on how we could help to harvest crops for fighting the undesired soil pollution from heavy metals associated with contamination. We here learned that polyamines such as putrescine as well as mycorrhiza glomus offer significant protective mechanisms against Cd-toxicity in pea through their protective role in plants, mediated by balancing the starch and lipid content. This study gave us insights into the efficient and balancing role of putrescine and mycorrhiza glomus for minimizing the damage done by Cd contamination. Importantly, we are confident that this defensive work could be extended to other heavy metals related toxicity but that needs a serious assessment in future work.

Acknowledgments

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Author Contributions

T.K., D.K, and P.K. designed the study, established the biochemical protocols, performed the experiments and collected the data analyzed and interpreted the data. P.K., D.K. and T.K. wrote the paper.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Figure 1

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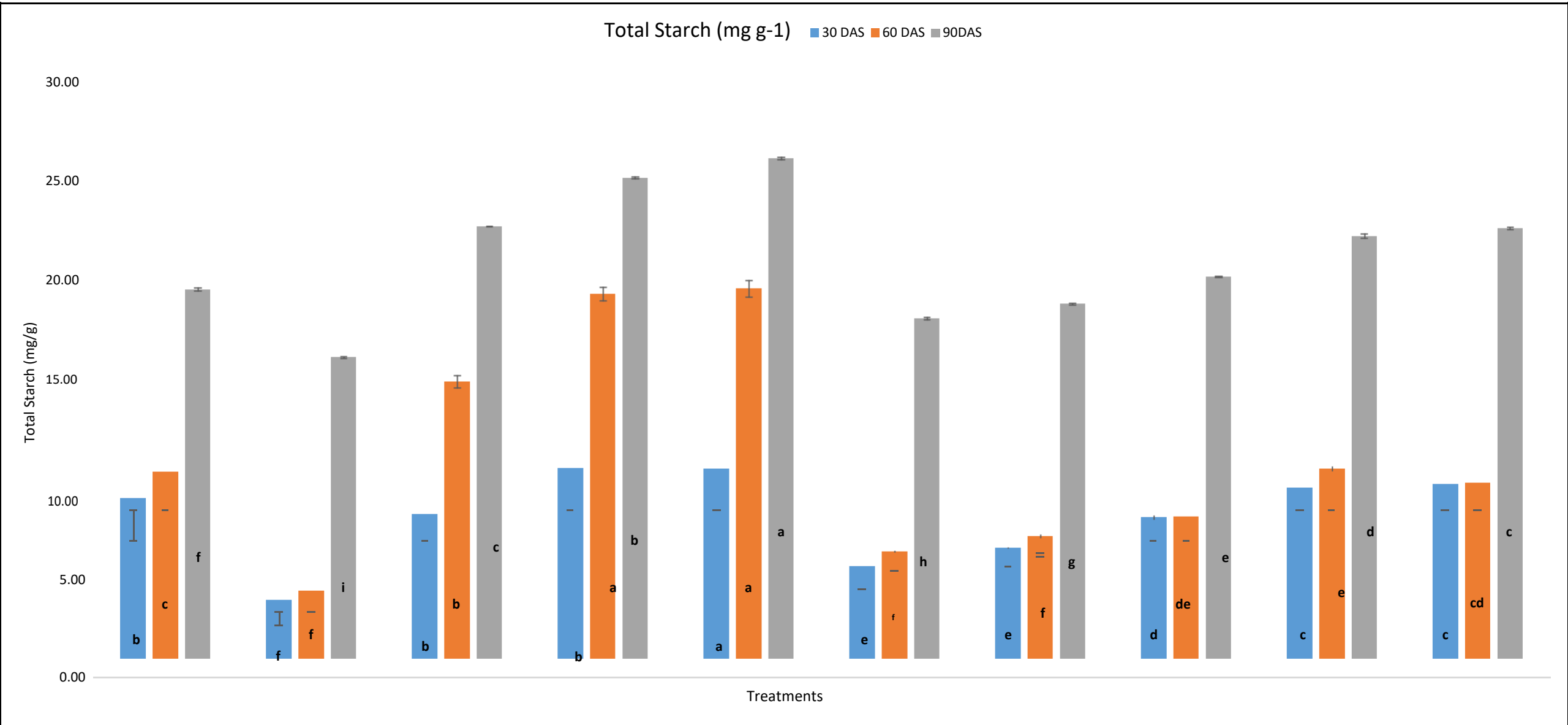
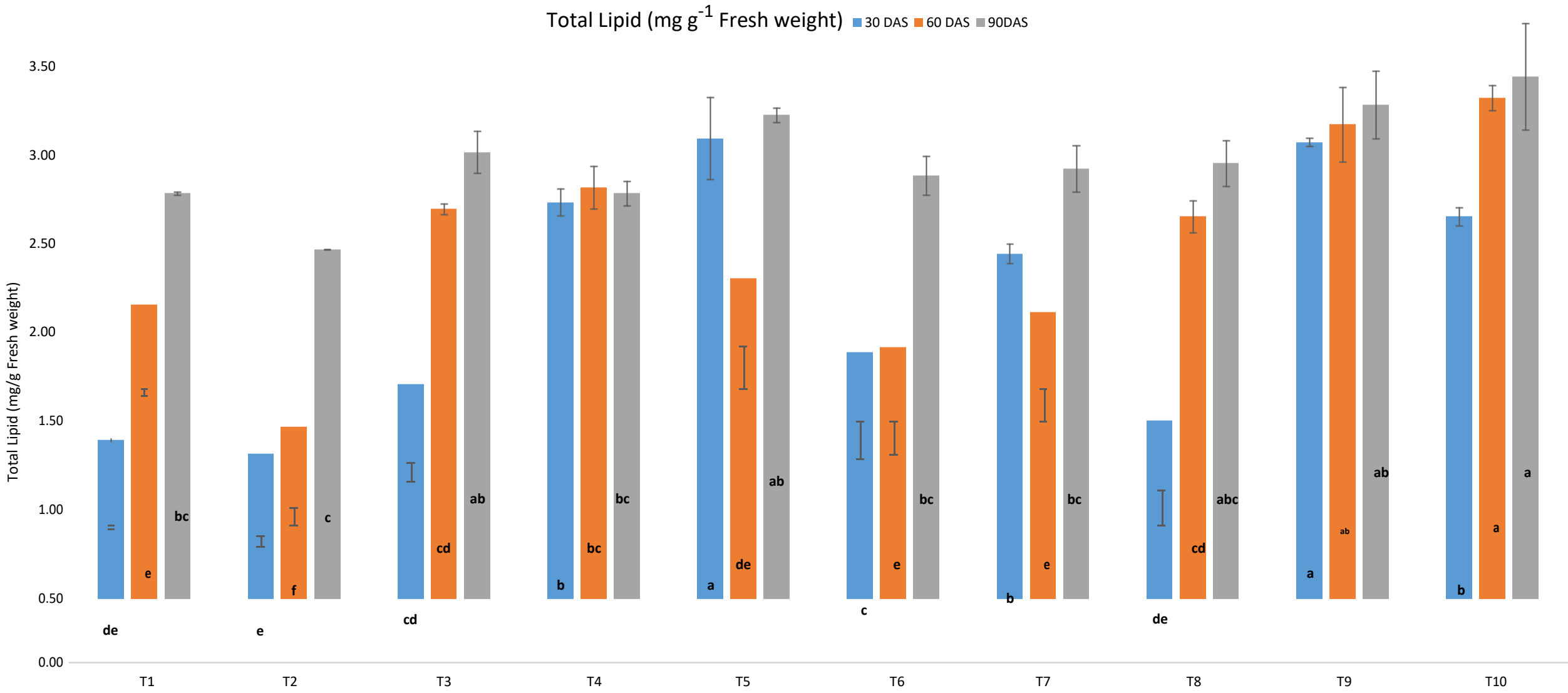


Figure 2


Total Lipid (mg g-1 Fresh weight)
30 DAS
60 DAS
90DAS
Total Lipid (mg/g Fresh weight)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
de
e
cd
b
a
c
b
de
ab
b

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Treatments	30DAS	60DAS	90DAS
T1	8.09^{b}±0.62	9.42^{c}±0.10	18.62^{f}±0.09
T2	2.96^{f}±0.33	3.41^{f}±0.13	15.19^{i}±0.05
T3	7.27^{b}±0.36	13.97^{b}±0.30	21.80^{c}±0.02
T4	9.60^{b}±0.18	18.38^{a}±0.35	24.25^{b}±0.06
T5	9.56^{a}±0.49	18.66^{a}±0.42	25.23^{a}±0.07
T6	4.66^{e}±0.20	5.39^{f}±0.04	17.15^{h}±0.05
T7	5.57^{e}±0.03	6.17^{f}±0.07	17.88^{g}±0.04
T8	7.11^{d}±0.08	7.17^{de}±0.11	19.25^{e}±0.11
T9	8.61^{c}±0.21	9.58^{e}±0.09	21.31^{d}±0.05
T10	8.80^{c}±0.27	8.87^{cd}±0.12	21.70^{c}±0.02

Treatments	30DAS	60DAS	90DAS
T1	0.9^{de}±0.009	1.66^{e}±0.02	2.29^{bc}±0.009
T2	0.82^{e}±0.029	0.97^{f}±0.06	1.97^{c}±0.002
T3	1.21^{cd}±0.053	2.20^{cd}±0.03	2.52^{ab}±0.12
T4	2.24^{b}±0.075	2.23^{bc}±0.12	2.29^{bc}±0.07
T5	2.6^{a}±0.232	1.81^{de}±0.10	2.73^{ab}±0.04
T6	1.39^{c}±0.107	1.42^{e}±0.11	2.39^{bc}±0.11
T7	1.95^{b}±0.054	1.62^{e}±0.09	2.43^{bc}±0.13
T8	1.01^{de}±0.099	2.16^{cd}±0.09	2.46^{abc}±0.13
T9	2.58^{a}±0.021	2.68^{ab}±0.21	2.79^{ab}±0.19
T10	2.16^{b}±0.052	2.83^{a}±0.07	2.95^{a}±0.30

Table 2: Total Lipid of pea after treatments

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Figure Captions:

Figure 1: Total starch content is estimated here with different experimental conditions. The different experimental conditions used as T1= Control; T2: Cadmium nitrate (100ppm); T3: Control + Mycorrhiza (*Glomus* sp., AMF); T4: Control + Putrescine (5 ppm); T5: Control + Putrescine (10 ppm); T6: Cadmium nitrate (100 ppm) + Putrescine (5ppm); T7: Cadmium nitrate (100 ppm) + Putrescine (10ppm); T8: Cadmium nitrate (100 ppm) + Mycorrhiza (*Glomus* sp., AMF); T9: Cadmium nitrate + Mycorrhiza (*Glomus* sp., AMF) + Putrescine (5 ppm); T10 : Cadmium nitrate (100 ppm) + Mycorrhiza (*Glomus* sp., AMF) + Putrescine (10 ppm). DAS= Days after sowing. Data are in the form mean± SEM. Significance at $P \leq 0.05$ using SPSS ver. 22.

Figure 2: Total lipid content is estimated here with different experimental conditions. Same experimental conditions as stated in Figure 1 also used here such as T1= Control; T2: Cadmium nitrate (100ppm); T3: Control + Mycorrhiza (*Glomus* sp., AMF); T4: Control + Putrescine (5 ppm); T5: Control + Putrescine (10 ppm); T6: Cadmium nitrate (100 ppm) + Putrescine (5ppm); T7: Cadmium nitrate (100 ppm) + Putrescine (10ppm); T8: Cadmium nitrate (100 ppm) + Mycorrhiza (*Glomus* sp., AMF); T9: Cadmium nitrate + Mycorrhiza (*Glomus* sp., AMF) + Putrescine (5 ppm); T10 : Cadmium nitrate (100 ppm) + Mycorrhiza (*Glomus* sp., AMF) + Putrescine (10 ppm). DAS= Days after sowing. Data are in the form mean± SEM. Significance at $P \leq 0.05$ using SPSS ver. 22.