Clarification of the referees are attached in the annexure

Clarification needed

Page no: 13
2.1 it is stated that soil samples collected from various kinds of plants and from different localities – list them.
2.1.1 P$^H$ correct it to pH
2.1.2 Estimation of Mycorrhizal colonization- describe the methodology
2.2 Estimation of growth rate- Describe the method and describe what the “etc” are.
Similarly specific methods adopted for each method has to be given in the thesis.

Page 14
RMD – This index has not been used in the thesis why then given in the methodology
2.3 The reference citation is not appropriate (Ref no127)
2.3.2 Estimation of P and K. Include name and make of the auto analyzer instead of describing it. Include principles.

Page 19
The setting up of the instrument is --------- nitrogen. What do you mean by this?
What do you mean by ‘ For 20 ppm the -----P?

Page 22
Calculation: describe the equation
Page 23: Calculation- where is the total volume of the sample in the calculation to get per g.
Page 26: item 10: correct ‘ Praline’ to ‘Proline’ why expressed as micro mole per g tissue see fig X page 163 and 164.
2.6.1: SOD: state whether SOD refers to total or Mn SOD or Zn SOD. Sample preparation needs to be given for all enzyme analysis and also describe the calculation of enzyme activity.
2.6.2-2.6.4 as above
2.7 Describe the principle and the make and essential components of GC
2.9: Statistical analysis: Use the term mean± not and average ±SD. Also explain the statistical methods used is it one way ANOVA with t test or any other post hoc test. The sources of important chemicals, medium and the make of instrument should be included.

Chapter 3:
Page 39: Dissecting microscope- provide details
All figures should be self –explanatory and should have legend.

Page 40 A total of 106 plant species: there should be a basis for selection of these plant species.
. For selection of host plant for inoculum production paddy has been selected.
  Is there any specific reason for selection? Other model plants such as Pisum sativum, Arabidopsis should have been tried.
. What was the yield of VAM by different techniques and viability for industry?

Chapter- 4
Values in the table are mean and not average.
Why the heavy metals not measured in the plants?
Why repeat the group-1 values repeatedly. It can be referred to the subsequent tables.
Why no statistical test done for Proline content?
What will be the impact of other heavy metals-Pb and Hg?
. RMD as an index should be used rather than providing the dry weight of the plants that too when the growth itself was effected.
. dry weight per unit of wet weight may be more useful than dry weight.
Clarifications

1. The detailed list of the plants is given in chapter 3 Table No: 1 page No: 41
2. pH corrected as mentioned ‘pH’
3. Detailed methodology has been given in chapter 3 page No: 40. estimation was done as per the Standard method (Ref. Giovanetti and Mosse, 1980)
4. "etc" refers to the No: of nodules and nodule weight. It is applicable only in chapter 5.
5. The index RMD (Relative Mycorrhizal Dependency) has been given in the methods and has been referred wherever it is needed. Eg: - page 65 line 8, page 73 line 5 etc. Values are given in tables VI, XI, LII and LXXV
6. A663- absorbance at 663 nm
   A645- absorbance at 645 nm
   A652- absorbance at 652nm
   V= Volume of the sample
   W= weight of the leaf sample taken
7. Total volume of the sample is 100ml. It is corrected in the equation
8. Praline is corrected to Proline. Micromole is changed to millimole.
9. Total SOD was calculated.
   Calculation of enzyme activity
   i) SOD was calculated by \( \frac{C-T}{C/2} \times \frac{2/3}{mg \text{ protein}} \)
      C= control (devoid of tissues, T= test
   ii) Catalase was estimated by
      \( \frac{T}{34} \times \frac{1000}{Aliquot \ taken} \times \frac{1}{Dil. \ factor} \times \frac{mg. \ protein}{mg. \ protein} \)
      O.D of ‘0’ time – O.D of 30” = Y, O.D of ‘0’ time – O.D of 60” = Z
      Average T= \( \frac{2Y+Z}{Z} \)
iii) GSH was calculated by

$$\frac{OD_{Test} \times \text{Conce. Std} \times \text{total volume of homogenate}}{O.D \times \text{Vol. Taken} \times \text{Wt. of tissue}} \times 100$$

iv) GST was estimated by using the following equation

$$A = \frac{\Delta OD}{\sum \times \text{N} \times \text{VH}}$$

$$\Sigma = 9.6 \text{ (extinction coefficient of CDNB conjugate)}$$

$$N = \text{No. of molecules of indicator converted per mole of substrate consumed}$$

$$\text{VH} = \text{volume of tissue extract}$$

$$\Delta OD = \text{change in optical density (absorbance/ minute).}$$

Leaf extract preparation for SOD

The tissue was homogenized in 0.25M sucrose and differentially centrifuged. Before estimating the activity of SOD, initial purification was done by precipitating the protein from the supernatant with 90% ammonium sulphate and after dialysis against 0.0025M tris HCl buffer, pH =7.4. The supernatant was used as the enzyme source.

Sample preparation for enzyme assays- The weighed sample of the plant material was extracted in saline buffer or in distilled water and centrifuged at 4°C in a cold ultracentrifuge. The supernatant was used for enzyme assay.

10. Nitrogenase- Principle

Acetylene is reduced to ethylene by nitrogenase. The ethylene produced is measured in a gas chromatograph (GLC) and the activity is expressed as 'n' mole ethylene produced for unit time per g dry weight.

Phosphatase- Principle

The enzyme phosphatase hydrolyzes p-nitrophenol phosphate. The released p-nitrophenol is yellow in colour in alkaline medium and is measured at 405nm, the optimum pH for acid and alkaline phosphatases are 5.3 and 10.5 respectively.
11. No specific reason for the selection of 106 plants. Random collection of plants from different localities in and around Kottayam and all the collected plants were included.

12. The reason for selection of paddy as the host
   i) As it is the chief food of keralites.
   ii) VAM needs a host plant for its multiplication
   iii) Paddy is a monocot plant and it has a very good fibrous root system.
   iv) Maximum root area for multiplication

13. VAM spores were multiplied in abundance when paddy was the host plant. 1gm dry soil contain 250 spores and stored it in 4°C for long viability. It could be stored for 3 months with out any loss but after 6 months 60% viable spores retained.

14. Values in the table mentioned are average of 6 values as suggested by one of the previous examiner.

15. The heavy metals in the plants were measured before taking the doses for study and it was found that 1000mg of Zn SO4/kg soil and 500mg of CdSO4/kg soil were not toxic to mycorrhizal plants. The shoot system (aerial part) contained only lesser amounts of Zn and Cd than their root system.

16. Group I (control group) values were repeated because all the treatments are considered separately. Each section needs the values of control for comparison.

17. Mean and SD were calculated and values are presented graphically.

18. Pb and Hg were not studied. But reports indicated same kind of results as in the case of Zn and Cd.

20. As the dry weight was used for calculating Relative Mycorrhizal Dependency (RMD) by most of the researchers in this area, we also followed the same procedure.