

I have reviewed the manuscript by Hatamzadeh et al. The manuscript still needs a major revision. I would suggest that authors should carefully read what they are writing as there are a lot of typographical mistakes. A lot of information is missing in the material and methods section. Authors should give a sound rationale for their study. A proper explanation is needed in some parts of the discussion and conclusion needs to be strengthened. Authors must recheck the results they have presented particularly in Table 3. The manuscript still needs attention on the English at some places. I would like to suggest some improvements to make the manuscript more clear and focused. The main revisions which need to be made are listed below.

### **Abstract**

Line 21: write “plants from asteraceae” instead of plants

Line 27: write “fungal endophytes” instead of endophytes

Line 31: write “activity of fungal endophytes” instead of activity

Line 32: write “Identification of endophytic fungi” instead of Identification

Line 32: write “DNA sequence” instead of sequence.

Line 33: Write “performed” or “achieved” instead of “done”

Line 33: did you mean "partial large subunit nrDNA (LSU)?"

Line 34: As you have written LSU in capital letters please do the same for TEF1 and TUB, or maintain consistency.

Line 34: Please maintain consistency. Write "TUB ( $\beta$ -tubulin)"

Line 39: Write “endophytic fungi from asteraceae members”

Line 40: delete “this”

Line 40: Please mention in the abstract that L-asparaginase enzyme has anticancer activity and therefore this study was conducted to bioprospect L-asparaginase producing endophytic fungi from members of asteraceae.

### **Introduction**

Line 45-46: Please provide reference for “Endophytes are microorganisms that reside inside plant tissues without causing symptoms and obvious negative effects to host plants.”

Line 60-62: Please provide some recent reference for “This enzyme is one of the most important biochemical therapeutic enzymes used in the treatment of various types of leukemia, such as acute lymphoblastic leukemia in children (McCredie & Ho, 1973).”

Line 64-65: Please provide reference for “Currently, L-asparaginase derived from *Escherichia coli* is the main source of asparaginase. And write “L- asparaginase” instead of “asparaginase”.

Line 66-67: Please explain how L-asparaginase derived from eukaryotes may induce less toxicity and reduced immune response?

Line 76: Are all the plants from asteraceae family studied here have anti-cancer property? If yes please provide the references.

## **Materials and Methods**

Line 92: What do you mean by “Four segments per plate” Write “Petri plate” or “Petri dish”. Also, as you have mentioned that 4 explants were used in one Petri dish. How many replicates of Petri dishes were used for per plant sample?

Line 96: “...pouring aliquots of the water...” how many  $\mu\text{l}$ ?

Line 96: why did you chose water agar medium for control, when you have used PDA for the isolation of fungal endophytes?

Line 97: “....incubated for 2 weeks.....” at what temperature?

Line 101-102: Please use an equation to show how colonization frequency was calculated.

Line 103: Please write “achieved using” instead of “done by”

Line 118: Write "Estimation" or "Evaluation" instead of “Measurement”

Line 119: please elaborate how the fungal isolated were inoculated in McDox broth.

Line 120: For the screening of L-asparaginase enzyme you have incubated fungal endophytes at  $26 \pm 2^\circ\text{C}$ . However, for the estimation of L-asparaginase the incubation was performed at  $36 \pm 2^\circ\text{C}$ . Please explain.

Line 121: Did you centrifuged or filtered the broth before pipetting 100  $\mu\text{l}$  of broth (crude enzyme)? As the broth may contain fungal debris.

Line 128-130: How did you quantifies L-asparaginase? Did you made any standard curve or used a formula? Also, how did you prepare the bank?

Line 135: “Molecular identification of the endophytic fungi” Please move this section up after isolation and identification of fungal endophytes.

Line 136: Please write “Endophytic fungal isolates” instead of “fungal isolates”

Line 139” You may write “molecular markers” instead of “loci”

Line 140: Write “(TUB)  $\beta$ -tubulin”

Line 140: Please mention make/model of PCR.

Line 140: Please check and correct: It should be “Table 2” “Table 1”

Line 143: Please mention Brand/company of Taq polymerase.

Line 148: Please mention make/model of UV transilluminator.

In material and methods add a section for statistical analysis and give details about statistical tests used in the manuscript.

## Results

Line 154: Please check period in “*A. altissima*” it must be “*A. altissima*”

Line 155: Write “*C. intybus*” in italics.

Results:

Line 157: Write “Eighty-five” instead of “Eighty five”

Line 157: “Eighty five endophytic fungal species belonging to Ascomycota and Basidiomycota were identified using morphological and molecular methods.” Please explain that why you identified only 85 fungal endophytes out of 827 isolates.

Line 158-162: Please correct the sentence to “Few endophytic fungi such as *Acremonium sclerotigenum*, *Alternaria burnsii*, *Bjerkandera adusta*, *Colletotrichum tanacetii*, *Epicoccum nigrum*, *Fusarium acuminatum*, *Paraphoma chrysanthemicola*, *Plectosphaerella cucumerina* and *Stemphylium amaranthi* showed wide distributions in the host plants and were isolated from most plants studied.”

Line 162: Place a comma after “Also,”

Line 165: Write “isolates from flower samples” instead of “isolates of flower samples.”

Line 170: Write B in capital letters in “Basidiomycetous”

Line 176: Write “endophytic fungal isolates” instead of “fungal isolates”

Line 177: Please correct spelling of “asparaginase”

Line 182: Correct spelling of “measured”

Line 181: Instead of writing “All six *Fusarium* species in this study had the ability to  
182 produce L-asparaginase,” you may write “All six *Fusarium* species in this study could  
produce produce L-asparaginase,”

Line 185: Write “Thirty-eight”

Line 189: Write “recorded to a range of” instead of “measured to range of”

Line 193: Write “the least” instead of “least”

Line 194: delete “statistically” As significant differences indicate that the difference is statistically significant.

Line 195: Write “ at 1%” instead of “1%”

## Discussion

Line 204: Please correct periods “*M. chamomilla*, *M. parthenium*, *A. triumfetii*, *A. altissima*, *A. millefolium*, *A. filipendulina* and *C. intybus*.”

Line 208-211: “Endophytic Basidiomycetes including *T. versicolor*, *B. adusta*, *T. biforme* and *S. commune* are white- rot fungi. Researchers have previously shown that most

Basidiomycetous endophytes are white-rot species (Oses et al., 2006; Thomas et al., 2008).

What do you want to explain with these statements? Please illuminate.

Line 214" Write "extrapolated" instead of "suggested"

Line 223: Write "recovered" instead of "isolated"

Line 220" Place a comma after "example,"

Line 226: Write "the treatment" instead of "treatment"

Line 232: Write "the ability" instead of "ability"

Line 236" Write "the low" instead of "low"

Line 242: Write "host plant examined in the present study" instead of "host plant we examined"

### **Conclusion**

Line 250: alternative source for this enzyme for what purpose? Elaborate.

The conclusion is very weak, please strengthen it.

### **Figure 1**

The name of all the fungal isolates in the phylogenetic tree must be in italics.

Please rewrite this sentence "A maximum parsimony phylogeny for *Fusarium proliferatum* from ITS" and also write full form of ITS in the caption.

What do you mean by "*Fusarium staphyleae* obtained from GenBank was treated"?

### **Figure 2**

Please correct this sentence to "Isolates showing high production of L-asparaginase"

### **Table 1**

Geographical coordinates of the studied areas in Northeast of Iran. Please submit this as a supplemental data.

Please mention about Geographical coordinates of the studied areas in line 86, with the make/model of GPS used. And write full form of GPS as well.

### **Table 3**

Please write "Colonization frequency of endophytic fungi"

In table 3 please make a row mentioning: "Colonization frequency" just above plant parts.

I would like to know why the total colonization frequency mentioned in the table is more than the actual total of colonization frequency from different plant parts? Please explain.

Also, the total of number of isolates is wrong: the total of 468+258+49+12 is 787 not 827 as mentioned in the table. Please check the total for colonization frequency as well.

### **Table 4**

Please write "L-asparaginase enzyme" instead of "enzyme" in the table.

Please mention in material and methods under statistics section that you have used LSD test for test for the quantification of L-asparaginase enzyme. Also write full form of LSD test.