Responses to the Reviewers’ Comments

The authors would like to thank the Reviewer’s for his/her constructive comments and suggestions that have helped us improve our manuscript. An extensive revision has been undertaken and incorporated all the corrections and suggestions raised by the Reviewer’s in the revised manuscript.

Reviewer 1:

Response: We are very glad that the Reviewer highly evaluated our manuscript, and provided constructive comments and suggestions that have helped us improve the quality of our manuscript.

Comment 1: Manuscript needs improvement.

Response: Thank you so much for your critical observation of our manuscript. To meet the Reviewer’s suggestion, the entire manuscript is revised and also read by a native speaker Prof. M. Quaterman from New Zealand.

Comment 2: In the Introduction and Discussion section, authors may mention recent very interesting book on selenium, which contains also chapter on selenium nanoparticles: Selenium / Michalke, Bernhard (Editor). Berlin, Germany: Springer International Publishing, 2018. pages 393-412; doi:10.1007/978-3-319-95390-8

Response: We would like to express our special thanks to the Reviewer for this important suggestion, with which we totally agree. Accordingly, the suggested reference is now incorporated along with the text in the introduction (L.53-54) and discussion section (L.317-319) of the revised manuscript.


Comment 3: Figures 2,6,7,8,11,12 are of very poor quality and should be improved

Response: We highly appreciate the Reviewer’s for this comment and suggestions. We have taken all our efforts to improve the resolution of the all the figures in the revised manuscript.

Comment 4: Figure 4 is not clear - figure caption does not explain properly what has been represented by this figure

Response: We would like to thank the Reviewer for this comment. The resolution of Figure 4 is improved and also revised the legends for easy understanding (P.6, L.228-230).
**Comment 5:** Authors claimed that SeNPs were characterized by sharp surface plasmon resonance (SPR) peak (lines 203-204), but SeNPs cannot have SPR due to selenium electronic configuration. There is simple method using protein corona formation to test the existence of SPR peak.

**Response:** We are extremely sorry for this mistake. The sentence “sharp surface plasmon resonance (SPR)” is now deleted from the revised manuscript.

**Comment 6:** Table 2 gives only mean values, but standard deviation should be provided. At least 3 replications should be included in these measurements.

**Response:** We again highly appreciate the Reviewer for his/her critical observation. As suggested by the Reviewer, the standard deviation is now incorporated in the table 2 (P.8). The table 2 in the manuscript represents the mean data obtained from three replicates. For the Reviewer information all the three replicates data is shown in the below table.

<table>
<thead>
<tr>
<th>SeNPs</th>
<th>Z-average size (nm)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Reading</td>
<td>2nd Reading</td>
</tr>
<tr>
<td>CF</td>
<td>98.5</td>
<td>103.2</td>
</tr>
<tr>
<td>CL</td>
<td>93.6</td>
<td>95.8</td>
</tr>
<tr>
<td>CW</td>
<td>93.2</td>
<td>99.9</td>
</tr>
</tbody>
</table>

**Reviewer 2**

**Response:** We are very glad that the Reviewer has positively evaluated our manuscript, and provided constructive comments and suggestions that have helped us improve the quality of our manuscript.

**Comment 1:** The experimental protocol is not well detailed and the results are not clearly presented.

**Response:** Thank you so much for your critical observation of our manuscript. As suggested by the Reviewer, the entire manuscript including the experimental protocol is revised. In addition, the manuscript is read by native speaker Prof. M. Quaterman from New Zealand.

**Comment 2:** Line 121. The authors should specify the type of fungi used to determine the antifungal activity of SeNPs. In addition, in the results section (line 185) the authors report experimental conditions (incubating the plates for 5 days at 22 ± 2°C) different from those described in the materials and methods section (Petri plates were incubated at 23 ± 2°C for seven days). The authors should explain this discrepancy.

**Response:** We highly appreciate the Reviewer for raising this comment. For the antifungal activity the fungus used is “P. grisea” (L.131). Sorry of this topographical error and also the
mistake. Actually, the PDA plates were incubated for seven days, on the fifth day at 23 ± 2°C the inhibition of the fungal growth was noticed and recorded (L.211-212).

Comment 3: Line 173. The authors use different fractions of T. atroviride broth culture to synthetize SeNPs but they do not report the characterization of the SeNPs obtained using these different fractions. Why? The authors should point out differences and similarities of SeNPs obtained using the different fractions. In addition, which fraction did the authors use to perform the tests described in the manuscript (antifungal activity, in vitro leaflet assay etc.)?

Response: Thank you very much for this inquiry. The synthesis was carried out with three different fractions namely: culture filtrate (CF), cell lysate (CL) and crude cell wall debris (CW). The preliminary characterization was performed using DLS to determine the Z average size and zeta potential of all nanoparticles. The results are shown in the following table:

<table>
<thead>
<tr>
<th>SeNPs</th>
<th>Z-average size (nm)</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>98.5</td>
<td>-49.3</td>
</tr>
<tr>
<td>CL</td>
<td>93.6</td>
<td>-43.7</td>
</tr>
<tr>
<td>CW</td>
<td>93.2</td>
<td>-48.7</td>
</tr>
</tbody>
</table>

Further, the nanoparticles derived from the three fractions were subjected to scanning electron microscopy to study their morphology. The obtained results clearly depicted a more uniform distribution of shape and size of SeNPs derived from CF fraction as compared to the fractions of CL and CW. Thus, for all the in vitro biological studies SeNPs derived from the culture filtrate (CF) fraction was considered (L.190-197).

For the Reviewer’s reference we have provided below all the three fractions data connected to FT-IR, SEM and HR-TEM.

Fig. 1: FT-IR spectrum of SeNPs derived from different fractions: (a) Culture Filtrate (CF); (b) Cell Lysate (CL); (c) Cell wall debris (CWD)
Comment 4: The results illustrated in Fig. 4 are unclear. The legend of this figure does not clearly explain the images. In addition, at line 192, the authors report “the antifungal activities of the synthesized nanoparticles increased proportionately with increasing concentrations of nanoparticles” but in Fig. 4 most images look similar. The authors should provide clearer images and better explanations of these results.

Response: We would like to thank the Reviewer for this comment. The resolution of Figure 4 is improved and also revised the legends for easy understanding (P.6, L.228-230). Further, to avoid confusion the sentence is revised as below:

“It was observed that high concentrations of SeNPs could effectively inhibit the spread of the infection under in vitro conditions” (L.219-220).