

I found the manuscript entitled “**Phytochemical production and antioxidant activity improvement of *Rhinacanthus nasutus* (L.) Kurz calli by in vitro polyploidization**” quite interesting, however, this work necessitates some improvements (listed below) that need to be addressed to better emphasize the contribution of this study to the current knowledge:

- Add some numerical results in abstract.
- Introduction is too short. Please, include more precise and recent literature in the manuscript.
- Line 64 informed: Several antimitotic agents can induce polyploidization such as colchicine. Colchicine can induce Polyploidization but it is not an antimitotic because it makes cells growth rapidly, In Pharmacology sciences antimitotic agents is a drug which acts against mitosis division and it has an anticarcinogenic properties.
Please, exclude the words (Anti mitotic agent)
- Line 71,72 informed: Therefore, polyploids increase productivity both morphologically and biochemically in plants.
Fortunately for you in this study, polyploidy gave promising results in terms of the required biochemical products, but it is a rule that cannot be generalized in the field of plant tissue culture because, invitro culture causes the plant to get rid of its usual behaviour in nature, and therefore it is very possible that compounds that it is accustomed to producing will disappear from its biological pathways. It is also very possible that we will be surprised by compounds that appear that the plant did not produce before if it was grown using traditional agricultural methods.
Therefore, I would like to delete the phrase (both morphologically and biochemically) from line 71.
- Line 93: In vitro polyploidy induction.
Material and Methods part is very well written
After Callus exposure to colchicine --- Directly informed: The calli were rinsed three times in 99 sterilized distilled water for 5 min and cultured on MS medium with 1 mg/L kinetin and 1 mg/L 100 2,4-D.
(What about the level of Agar, was a shaker used at this stage? or was the growing medium solid or semi-solid, and what was the level of agar added?)
- Line 103: Flow cytometry analysis (Please add reference).
- Line 115: Preparation of *R. nasutus* callus extracts (Please add reference).
- Tables need captions.
- Please support your data with Plant culture photos to clear the differences between plant treatments even if, a previous study has been done in this regard.
- More accurate material identification techniques such as GC mass or HPLC are preferred especially since a great effort has been made to reach diploidization, even tetraploid to have a greater opportunity to define the resulting materials more accurately.