Dear editor in Chief

Here are rebrutal letter regarding reviewer comments to our manuscript entitle: **Growth of Kaffir Lime (*Citrus hystrix* DC) Cell Line After Yeast Elicitation Using *Saccharomyces cerevisiae* and Commercial Baker's Yeast**
I give highlight in purple and green so you can easily find the revised part in manuscript.

Reviewer(s)' Comments to Author

Reviewer A

1. General comments:

   a. The research is interesting in term of using readily available commercial baker's yeast in callus elicitation.

      Author Revision:

      We've edited the title into: "Growth of Kaffir Lime (*Citrus hystrix* DC) Cell Line After Yeast Elicitation Using *Saccharomyces cerevisiae* and Commercial Baker's Yeast"

   b. It is suggested to remove the yeast morphology and growth profile comparison because it's incorrect and is not in line with the main question of this manuscript.

      AUTHOR REVISION:

      This study contain 2 main topics: firstly we want to reveal that commercial baker's yeast (Fermipan) has ability to be used as an elicitor candidate for kaffir lime cell suspension culture, as well as *Saccharomyces cerevisiae*. Secondly, we determined the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period. Therefore, data about yeast morphology and its growth profile is important to be provided in this manuscript.

2. It is better to include "commercial baker's yeast" in the tittle, because the main purpose of this study was to compare the use of pure *S. cerevisiae* with Fermipan yeast.

   Author Revision:

   We've edited the title into: "Growth of Kaffir Lime (*Citrus hystrix* DC) Cell Line After Yeast Elicitation Using *Saccharomyces cerevisiae* and Commercial Baker's Yeast"

3. The term "4th generation" is incorrect to be used here. It is better to called it "4th subculture".
   AUTHOR REVISION:

   "*S. cerevisiae* and Fermipan subculture was carried out four times to obtain fourth - subculture (G4). Futhermore we deleted sentence about

4. The family Saccharomycetaceae contains members with similar cell morphology. Hence, that character cannot be used to identify the yeast. Biochemical method is the cheapest

method can be used. DNA sequencing (ITS or D1/D2) can also be used although more expensive. I suggest the author revise this part.

AUTHOR REVISION:

This data showed that Fermipan is referred to as an elicitor candidate for kaffir lime cell suspension culture . However, the character cannot be used to identify and compare the yeast because the Family Saccharomycetaceae contains members with similar cell morphology, therefore further identify with more accurate methods such as Biochemical and DNA sequencing methods is needed.

5. Please add error bars to each data point (figure 5)

AUTHOR REVISION:

We've revised figure 6 in masnuscript according to reviewer's sugestion.

Reviewer C

1. Morphology refers to kaffir lime cell line or fermipan? (line 18/19)

AUTHOR REVISION:

Morphology refer to fermipan. We've edited the sentence into:

"We observed the morphology of Fermipan and measured the growth of Fermipan from G0 to the G4, compared to *S. cerevisiae*."

2. *yeast* : Fermipan and *S. Cerevisiae?* (line 24, "using yeast")

AUTHOR REVISION: "On the other hand, after elicitation by using yeast (*S. cerevisiae* and Fermipan) and being subcultured, the growth of elicitated kaffir lime line cells had the same pattern as the control group, but the cell density of the control group was higher than the elicitated group."

3. *Elicitor is **abiotic** or **abiotic** compound.* Did you mean **biotic** or **abiotic?**

AUTHOR REVISION: "Elicitor is biotic or abiotic compound that induces synthesis of other specific compounds used in the defense mechanism of plants (Murthy et al. 2014; Ramirez-Estrada et al. 2016)."

4. The word "this compound" refers to which sentence? Physical factors (e.g. cold shock, UV light, and high pressure) are not compounds (line 38).

AUTHOR REVISION: "These biotic and abiotic elicitors trigger enzymatic activities in plant stress responses (Gueven 2003; Ramirez-Estrada et al. 2016).

5. (*easy to obtain*) contradictive with the sentence in line 54 (*not being sold freely, impractical, and difficult to carry from one place to another because it must be in aseptic condition*)

AUTHOR REVISION: “Furthermore, the use of *S. cerevisiae* as an elicitor has several advantages such as it is easy to grow, short life cycle, can grow at low pH, and is safe for health because it is not toxic (Sitinjak et al. 2000).”

6. Is there any evidence that kaffir lime cell suspension culture can produce terpenoid? (line 67-68)

   AUTHOR REVISION:

   Yes, we had data about it (unpublished data**).**

7. *Secondly, we determined the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period in order to* ***maintain the stability of the kaffir lime cell line compounds.*** Can this study determine the stability of compounds in cell cultures, when no analysis of secondary metabolite content in cell cultures is performed? (line 88-90)

   AUTHOR REVISION:

   We’ve revised all sentences about terpenoid synteshis, since this paper mainly focus on determination about the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period. “

8. What does clean conditions mean in this sentence? (“comletely clean” line 108).

   AUTHOR REVISION: the sentence was edited into “Seeds were removed from the fruit then sterilized using chlorox 5.25% for 5 min.”

9. Is it also done with culture of *S. cerevisiae*? (line 121-122)

   AUTHOR REVISION:

   “*S. cerevisiae* and Fermipan subculture was carried out four times to obtain fourth - subculture (G4). “

10. What does this sentence mean? Maybe this sentence is more proper *: Elicitation was carried out by adding Fermipan (5 ppm, 10 ppm) and S. cerevisiae (10 ppm) into 50 ml of cell suspension culture.* (line 152)

    AUTHOR REVISION:

    Thanks for the suggestion. We’ve change the sentence into:

    “Elicitation was carried out by adding Fermipan (5 ppm, 10 ppm) and *S. cerevisiae* (10 ppm) into 50 ml of cell suspension culture.”

11. Does the same morphology indicate the same activity? Is there a reference that says that? A compound can be used as an elisitor if it is able to increase the production of secondary metabolites. Elisitors are often specific to certain types of secondary metabolites. A culture is said to be elicitated if the production of secondary metabolites increases or changes with the addition of elisitors. Can it determine the elisitation activity of a compound in a cell

culture without analyzing the metabolite content of the cell culture's secondary metabolites? It may be more appropriate if fermipan is referred to as an elisitor candidate for kaffir lime cell suspension culture. (line 194-195)

AUTHOR REVISION:

"This data showed that Fermipan is referred to as an elicitor candidate for kaffir lime cell suspension culture . However, the character cannot be used to identify and compare the yeast because the Family Saccharomycetaceae contains members with similar cell morphology, therefore further identify with more accurate methods such as Biochemical and DNA sequencing methods is needed."

12. Does that mean that there are cells that do not survive because the presence of elisitor (elisitor causes cell death)? Please add references that support this statement. (line 270-272)

AUTHOR REVISION:

Yes, there were dead cells after elicitation. We've added this sentence.

"High doses of elicitors can induce a hypersensitive response that causes cell death (Namdeo 2007), so we assume that cells that can survive are cells that are resistant to biotic stress and are expected to be able to produce high levels of metabolites."

13. Is there a reference that says that? (line 329-331, tentang subkultur sel lini dapat mempertahankan senyawa)

AUTHOR REVISION:

"Subculture of line cells with a certain period is one method to maintain cell viability to produce bioactive compounds. According to a study conducted by Sierra et al. (1992) succeeded in stabilizing alkaloid compounds in two cell line cultures of the *Tabernaemontana divaricata*. The highest production of alkaloids in the 4th after the change of the medium and the growth remains stable during 30 subculture with a subculture interval of every 9 days. The other study was found in the production of betaxanthins in callus culture of *Beta vulgaris* L. var 'Dark Detroit'. Production of betaxanthins in callus cells line of the *Beta vulgaris* increased 1.8-fold after 48 subcultures with subculture intervals every 14 days (Trejo-Tapia et al. 2008). As well as the stability of synthesizing verbascoside in suspension cell line culture of *Buddleja cordata* Kunth after being subcultured for 5 continuous years (Arano-Varela et al. 2020)"

14. Does the same morphology and growth curves indicate the same activity? (line 338-339)

AUTHOR REVISION:

According to that data, we assumed Fermipan had ability as an elicitor candidate for kaffir lime cell suspension culture."

Reviewer D

1. (General comments) This paper is interesting, however major revision must be done.

   a. Title is misleading. The experiment was about growth comparison of cell suspension culture of lime by addition of pure yeast and commercial yeast. This must be written in title.

   AUTHOR REVISION: "Growth of Kaffir Lime (*Citrus hystrix* DC) Cell Line After Yeast Elicitation Using *Saccharomyces cerevisiae* and Commercial Baker's Yeast."

   b. This paper can not only terminate to the growth of the cells, but also need to continue to analyse the main product (secondary metabolite product of lime cell).

AUTHOR REVISION:

This paper mainly focus on determination about the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period. "

2. The main idea is to increase the secondary metabolite production od lime cell cultures by elicitation by yeast. The first sentence must be about wgy lime cell culture need elicitor

   AUTHOR REVISION: "Kaffir lime cell culture requires elicitor to increase the production of secondary metabolites. *Saccharomyces cerevisiae* is one of the elicitors that is used to increase secondary metabolite compounds such as terpenoids."

3. This is in complete sentence, several impractical of what? Please mention (line 14)

   AUTHOR REVISION: "However in its use, the pure culture of *S. saccharomyces* are difficult to carry because it must be in aseptic conditions."

4. Please rewrite in better order. Firstly, about the important of lime cell culture, followed by elicitation, followed by types of elicitors, folloed by s. cereviciae as elicitor. Give many example how to increase in cell culture, followed by why you need Fermipan, finally the objective of the study (**in introduction**)

   AUTHOR REVISION: "Extract of kaffirr lime leaf has potential as an anticancer (Tunjung et al. 2015). However the use of extract from the nature has several obstacles such as over exploitation of kaffir limeleaf and the production of bioactive compounds is strongly influenced by environmental conditions. One methods for producing bioactive compounds can be done by tissue culture techniques (Bourgaud et al.2001). Unfortunately, the type and level of bioactive compounds in callus extract of kaffir lime were less than the leaf extract. Therefore, kaffir lime cell culture requires elicitor to increase the production of bioactive compounds.

Elicitor is biotic or abiotic compound that induces synthesis of other specific compounds used in the defense mechanism of plants (Murthy et al. 2014; Ramirez-Estrada et al. 2016). Biotic elicitors consist of living organisms such as fungi, bacteria, and herbivores, while inorganic compounds, heavy metals, pesticides, detergents, or physical factors (e.g. cold shock, UV light, and high pressure) are examples of abiotic elicitors. These biotic and abiotic elicitors trigger enzymatic activities in plant stress responses (Gueven 2003; Ramirez-Estrada et al. 2016).

S. cerevisiae is yeast that can increase the content of terpenoid compounds in some plants. Treatment by *S. cerevisiae* with a concentration of 1.5% for 72 hwas able to increase the content of gymnemic acid by 9.3 fold - in the suspension culture of *Gymnema sylvestre*. (Chodisetti et al. 2013). Furthermore, *S. cerevisiae* increase ajmalicine content in cell aggregate culture of *Catharanthus roseus*. Ajmalicine content was increased 25.288 ± 0.102 jig/g DW after being treated with *S. cerevisiae* with a concentration of 0.5% for 24 h(Ratnasari et al. 2001). Moreover, according to Pereire et al. (2007) the production of triterpenes was increased after added of *S. cerevisiae* to the cell suspension culture of *Tabernaemontana catharinensis.*

Furthermore, the use of *S. cerevisiae* as an elicitor has several advantages such as it is easy togrow, short life cycle, can grow at low pH, and is safe for health because it is not toxic (Sitinjak et al. 2000). On the other hand, in its use, *S. cerevisiae* has several disadvantages includingexpensive , not being sold freely, impractical, and difficult to carry from one place to another because it must be in aseptic conditions. Therefore we need another type of yeast elicitor that has the same ability as *S. cerevisiae*. Fermipan is a commercial baker's yeast consisting of *S. cerevisiae* and has several advantages, including being easy to find, easy to use, easy to carry because it is in powder form, and has affordable price. In the future research Fermipan will be an elicitor candidate for kaffir lime cell suspension culture..

Kaffir lime (*Citrus hystryx* DC) shows potency to be used as traditional medicine for several diseases such as cancer. In our previous study, we found that terpenoid is detected in first subculture 35d (control group) and in callus preserve in 4°C of kaffir lime callus. The type of terpenoids was squalene and geranyl acetate, whereas Geranyl linalool was found in kaffir lime callus preserve in 4°C with alginate encapsulation. Furthermore, some compounds act as anti-cancer also detected in preserved callus such as Lauric acid, palmitic acid, stearic acid, 1-Decanol, undecylenic acid, oleic acid, 2H pyran-2-one, octadecane, 1-Hexcosanol, Hexane, Methane, dodecane, tetracosane, 2 Decenoid acid, and 3-dodecane (Fajarina et al. 2021).

5. Fermipan is also yeast isn't ? Please give clear explanation between what you mean by "yeast' ang Fermipan. Both are yeast. Please do not confusing, must be clear. Do you mean laboratory or pure yeast (produced by certain chemical company) compare to commercial yeast which is available in the traditional market? {;ease make clear

AUTHOR REVISION:

Thank for the suggestion. We've edited all the sentences into: pure culture of yeast and commercial baker's yeast.

6. Which compound? Unclear (line 90)

AUTHOR REVISION:

We've revised all sentences about compound or terpenoid syntesis, since this paper mainly focus on determination about the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period. "

"Therefore, there are 2 objectives in this study. Firstly, we analyzed the ability of commercial yeast to be used as an elicitor. Secondly, we determined the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period".

7. Experimental design and English are poor (line 91, mengomentari materials and methods secara keseluruhan)

AUTHOR REVISION:

We've edited all the material and methods section.

8. Please also include the origin of S.cerevisiae

AUTHOR REVISION: . The pure cultures of S. cerevisiae were taken from Pusat Studi Pangan dan Gizi Universitas Gadjah Mada whereas Fermipan powder was bought from super market.

9. This the same as subtitle 2.1. Please rearrange (line 98)

AUTHOR REVISION: "Kaffir lime fruit was obtained from kaffir lime orchards in Kaliduren Village, Candirejo, Borobudur, Magelang Regency, Central Java. The fruit used wasfresh, the diameter of the fruit wasapproximately 5-6 cm, the length and width of the seed are approximately 0,9 cm and 0,42 cm, respectively. The origin of pure S. cerevisiae from Pusat Studi Pangan dan Gizi Universitas Gadjah Mada and Fermipan geting from tradisional market."

10. Ref (line 100, mengometari refresensi penggunaan medium kultur kalus)

AUTHOR REVISION: "The basal medium used is Murashige and Skoog (MS). Preparation of culture medium, each stock solution with a certain dose is added with myo-inositol, sucrose (30 g/l), agar (6 g/l), 1N HCl/KOH 1 N, and distilled water with 2 ppm 2,4-D. After the medium is boiling, the medium was poured into culture bottles and sterilized using an autoclave at 121^{o}C for 15 min (Damayanti et al. 2020)

11. Unnecessary , please delete (line 100-104, mengomentari penulisan bahan mikro dan makro nutririent MS)

AUTHOR REVISION:

We've deleted the sentence according to reviewer suggestion.

12. What concentration (line 105)

AUTHOR REVISION: "The concentration was 2 ppm of 2,4-D"

13. Sugar, agar, how much

AUTHOR REVISION: "sucrose (30 g/l), agar (6 g/l),"

14. Na-hypochlorite? I do not think that you can find Clorox. (line 109)

AUTHOR REVISION:

We've edited the sentence according to reviewer suggestion.

15. What does this mean? (line 109, mengometari kata "complately clean

AUTHOR REVISION: "Seeds were removed from the fruit then sterilized using Sodium hypochlorite 5.25% for 5 min. After that, the sterilizing solution was removed and the seeds were washed with distilled water 2 times for 5 min. After sterilization, explants were transferred to sterile petri dishes lined with filter paper (Damayanti et al. 2020)."

16. Please make ot clear, seeds or endosperm? Whole seeds or cut endosperm? {lease see Fig A. Inconsistent.

AUTHOR REVISION: "Cut sterile whole seeds were grown on MS solid medium containing 2 ppm 2,4-D. The kaffir lime seeds that were used for explants were part of the endosperm.

17. Solid? (line 115, mengomentari bentuk medium MS)

AUTHOR REVISION: "..solid medium"

18. What does this mean, please mention for how long? 25-30 days? Must be précised (line 117)→berdasarkan referensi

AUTHOR REVISION: Seeds were maintained stored in the incubation room under dark conditions until the stationary phase that is 25-30 days (G0) and subcultured to a new medium for 25-30 days (G1). This protocol was referred to previous study by Damayanti et al. (2020).

19. The design is poor. It should also have G4 of pure yeast to be consistent. The choice of G4 also unclear. Why not G2 and G3? Suddenly G4? (line 121)

AUTHOR REVISION:

This study is the first scientific research using Fermipan as an elicitor in tissue culture. We used Fermipan in the G4 subculture because we wanted to ensure its viability and growth stability. Fermipan contains S. cerevisiae and an emulsifier (sorbitan monostearate E491)

which can affect yeast growth. If the yeast growth in Fermipan has stabilized at G4, we assumed that yeast in Fermipan able to grow well and can be used as an elicitor.

20. 2.2.3. 2.2.4 and 2.2.5 can be combine as one subtitle. Make new subtitle (line 126)

AUTHOR REVISION: We've edited the sentence according to reviewer suggestion.

21. What does this mean? Do you mean all cultures? (line 127)

AUTHOR REVISION: "*S. cerevisiae* G4 and Fermipan G4 was grown in PGY liquid media for 24 hours. After 24 hours, 1 ml of yeast was taken from the liquid medium for morphological observations using a microscope ."

22. Is this G1 and G4, please mention (line 133, pengukuran pertumbuhan yeast)

AUTHOR REVISION: "One ose of one week old pure cultures of S. cerevisiae and G4 fermipan were inoculated into 10 ml of PGY liquid medium then incubated at room temperature without shaking. Cells were harvested at 2 h to 24 h time intervals to obtain growth curves. The cell number was calculated using a spectrophotometer at a wavelength of 660 nm. Measurements were carried out with 3 replicates. The number of cells that have reached the beginning of the stationary phase are used as an elicitor.

23. This is unnecessary. Use only cell number (line 136)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.

24. Normally at 660 nm

AUTHOR REVISION:

We've edited the sentence according to reviewer suggestion.

The cell number was calculated using a spectrophotometer at a wavelength of 660 nm.

25. Make it short : Yeast elicitation (line 150)

AUTHOR REVISION: We've edited the sentence according to reviewer suggestion.

26. G1 and G4? (line 155-156)

AUTHOR REVISION: "G4"

27. No need subtitle, combine with 2.2.7 directly (line 159)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.

28. Please, rewrite, English is confusing (line 160-170)

AUTHOR REVISION: After harvested, cell lines were filtered using a 100 µm nylon filter and washed using MS liquid medium to ensure that there was no yeast contamination inside cell suspension. This step is important to make sure that the elicitation period finished. The filtered cells were subcultured into 40 ml of MS+2 ppm of 2,4-D liquid medium. The growth of cell lines and control group were measured using a Neubauer hemacytometer for 27 days.

29. You did not mention the price, how to compare? (line 175-176)

AUTHOR REVISION: "(one tube pure culture's price of S. cerevisiae is Rp 500.000,00 while one sachet of Fermipan is Rp 5.000,00).

30. Our aim was…. Please use better word (line 177)

AUTHOR REVISION: *S. cerevisiae* was used as a control because this type of yeast is commonly used as an elicitor. Therefore, in this study, we compared these two types of yeasts to see their ability as an elicitor.

31. Why you show these photos for pure yeast up to G4, but you do not use G4 for elicitation? (fifure 1) This the weakness of this experiment.

AUTHOR REVISION: We use G4 of yeast for elicitation.

32. Please explain why you need to have stability culture up to 4th generation

AUTHOR REVISION: This study is the first scientific research using Fermipan as an elicitor in tissue culture. We used Fermipan in the G4 subculture because we wanted to ensure its viability and growth stability. Fermipan contains S. cerevisiae and an emulsifier (sorbitan monostearate E491) which can affect yeast growth. If the yeast growth in Fermipan has stabilized at G4, we assumed that yeast in Fermipan able to grow well and can be used as an elicitor.

33. It must also need phothographs as you show in Fig 1. (line 183)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.

34. Fig must be clear, no diameter and area written inside the photographs. Please delete. Only scale bar put inside picture (figure 2)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.

35. To show this, it should have some clear and closed-up photos with scale bar. (line 190)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.

36. This is not the way to determine the size. Please do the proper method. Take certain vol. measure, for instant, 100 cells, used statistic analysis to see the differences from G0 to G4. (line 190-191)

AUTHOR REVISION: We've edited the data according to reviewer suggestion.The average of cell size showed at table 1.

37. Unclear, must be close up photo (line 193)

AUTHOR REVISION: We've edited the data according to reviewer suggestion (figure 2 in manuscript).

38. Must have better data, not from photos (line 196)

AUTHOR REVISION: the average of cell size showed at table 1

39. This conclusion is incorrect, misleading. Fermipan can also be used as elicitor. To substitute meaning many things, not only based on the shape and growth. You have to prove whether the 2 different yeast can increase the metabolite product of lime cell culture. (line 197-198)

AUTHOR REVISION: This study contain 2 main topics: firstly we want to reveal that commercial baker's yeast (Fermipan) has ability to be used as an elicitor candidate for kaffir lime cell suspension culture, as well as Saccharomyces cerevisiae. Secondly, we determined the growth of kaffir lime cell lines after yeast elicitation to obtain the best subculture period.

We've data about 2 type of yeast can increase the bioactive compounds, but we separate them into different paper.

40. Delete, this already explained in methods (200-201)

AUTHOR REVISION: We've deleted the sentence according to reviewer suggestion.

41. Legend of Fig is G5? Supposed G4? (line 203, figure 3)

AUTHOR REVISION: We've edited figure 3 according to reviewer suggestion.

42. Do you mean he right time? (line 207)

AUTHOR REVISION: "This peak period is the best timing to harvest the yeast cells for the elicitation process."

43. Is Klis et al use yeast as elicitor? Please have more ref, what the right phase of yeast use for elicitor. Give example in some plant cell culture (209-211)

AUTHOR REVISION: No, it is not. Klis only describes the condition of yeast cells during the stationary phase.

44. What the relation with elicitation? (mengomentari line 209-211)

AUTHOR REVISION:

"Sigmoid curves were achieved when both yeasts reached their maximum growth (Fig. 3; see 16 h incubation period), at the 16th hour, indicated by the cells reached their stationary phase. This peak period is the best to harvest the yeast cells for the elicitation process. In this phase the yeast is at the highest growth, the yeast cell wall is well-formed and thicker, have a considerably higher turgor pressure compared to exponentially growing cells (Klis et al. 2002). At the stationary phase, the rate of yeast cells divisions are declining thus extra energy is allocated to form a compact cell wall structure (Aleu et al. 1999). According to Chen and Chen (2000), the response of plant cells to elicitors is directly related to the composition of the yeast cell wall, especially glucan, which is able to be recognized by plant cells as stress so that plant cells will respond by producing secondary metabolites."

45. This is common knowledge of yeast used for elicitor. Please rewrite with better English. (line 212-215)

AUTHOR REVISION: "Sigmoid curves were achieved when both yeasts reached their maximum growth (Fig. 3; see 16 h incubation period), at the 16th hour, indicated by the cells reached their stationary phase. This peak period is the best to harvest the yeast cells for the elicitation process. In this phase the yeast is at the highest growth, the yeast cell wall is well-formed and thicker, have a considerably higher turgor pressure compared to exponentially growing cells (Klis et al. 2002). At the stationary phase, the rate of yeast cells divisions are declining thus extra energy is allocated to form a compact cell wall structure (Aleu et al. 1999). According to Chen and Chen (2000), the response of plant cells to elicitors is directly related to the composition of the yeast cell wall, especially glucan, which is able to be recognized by plant cells as stress so that plant cells will respond by producing secondary metabolites."

46. None used this. Please delete. Elicitor of yeast always death, never alive. (line 218-219)

AUTHOR REVISION: We've deleted the sentence according to reviewer suggestion.

47. Cell culture? Cell suspension? (line 221, "cell line"?)

AUTHOR REVISION: "Cell lines are the cells that can survive to biotic stress and are expected to be able to produce high levels of metabolites."

48. This is general statement? Please use ref. (line 222-223)

AUTHOR REVISION: "A Friable callus is needed as raw material for cell suspension. The friable texture of the callus facilitates the separation between cells into a single cell in cell suspension culture (Damayanti et al. 2020)."

49. Delete. This is method (line 224-225)

AUTHOR REVISION: We've deleted the sentence according to reviewer suggestion.

50. Please refer to which data? (line 231-232)

AUTHOR REVISION: “Seeds were maintained stored in the incubation room under dark conditions until the stationary phase that is 25-30 days (G0) and subcultured to a new medium for 25-30 days (G1). This protocol was referred to previous study by Damayanti et al. (2020).”

51. Is Damayanti doing elicitation? (line 236-237)

AUTHOR REVISION: In paper Damayanti et al. (2020), we did not doing elicitation yet. But Damayanti et al had done elicitation on the other paper (not published yet).

52. How do you know? Use data or ref (line 237-238)

AUTHOR REVISION: “The time of cell suspension subculture was 16 days. On the 16th day, the new cell growth entered the stationary phase, so that before entering the death phase, the cell suspension was subcultured (Damayanti et al. 2020).

53. Please give general title, followed by what A, B, C etc. Fig A is meaningless, must be whole endosperm. Figs B, C and D must have scale bar, and state the name of culture medium. Please also place Figure after explanatory paragraph

AUTHOR REVISION: We’ve edited figure 4 and 5 according to reviewer suggestion.

54. Seeds or endosperm? {lease make it clear (line 240)

AUTHOR REVISION: “Figure 4. showed the explants that are used in this study are kaffir lime seeds. The kaffir lime seeds that were used for explants were part of the endosperm.”

55. No need photo. Delete (line 244-245)

AUTHOR REVISION: We’ve edited figure 4 according to reviewer suggestion.

56. Give ref to give example in other plants also use endosperm for explant of cell culture (line 246

AUTHOR REVISION: “Friable endosperm callus can used as raw materiall for cell suspension culture. According to Pasitvilaiturm & Pankasemsuk (2012) cell suspension culture of J*atropha curcas* L. can grow and produce oil.”

57. Ref (line 251)

AUTHOR REVISION: “The friable callus is needed as a raw material for making cell suspension ((Damayanti et al. 2020).”

58. It is difficult to see single cell aggregate in Fig. D. This is misleading. Please revise Fig D to show single cell aggregate. Fig D is cell suspension culture appearance in a flask

AUTHOR REVISION: We’ve edited figure 5 according to reviewer suggestion.

59. Give example (line 254-256)

AUTHOR REVISION: “It was found in mangosteen (*Garcinia mangostana* L.) culture, the production of secondary metabolites in callus treated with MeJa or CH was lower (21 metabolites) than that of cell suspension (34 metabolites) (Jamil et al. 2018).”

60. Give example of what plant cell cultre (line 258-259)

AUTHOR REVISION: “Agitation or shaking in *Jatropha curcas* L cell suspension cultures can increase aeration to maintain cell viability during the incubation period (Dwimahyani 2007).”

61. You did not determine the sec met production. You only check the growth. This statement is not relevant (line 264-268)

AUTHOR REVISION: According to some studies, subculture of cell line suspension is one of the methods that could stabilize synthesis of secondary metabolite. To get the best time for kaffir lime cell line subculture, we need to measure kaffir lime cell line growth after elicitation.

62. A and B are the same data, Only different presentation based on Abs and cell density. Please delete A, use B only. Place the figure after explanation

AUTHOR REVISION: We’ve edited figure 6 according to reviewer suggestion.

63. Use B only. Delete A. Data must be the same (line 272-273)

AUTHOR REVISION: We’ve edited figure 6 according to reviewer suggestion.

64. Ref (line 274-275)

AUTHOR REVISION: “High doses of elicitors can induce a hypersensitive response that causes cell death (Namdeo 2007), so we assume that cells that can survive are cells that are resistant to biotic stress and are expected to be able to produce high levels of metabolites.”

65. This is not relevant, you did not have data on sec met product (275-279)

AUTHOR REVISION: “This is because elicitation can screen cells and form cell lines. High doses of elicitors can induce a hypersensitive response that causes cell death (Namdeo 2007), so we assume that cells that can survive are cells that are resistant to biotic stress and are expected to be able to produce high levels of metabolites.”

66. Please take a look at Fig 5, at Day 0 you did not start with the same amount of culture. You have to change the data by increase of growth. So, you start with the same amount which is at zero.

AUTHOR REVISION: We’ve edited figure 5 according to reviewer suggestion.

67. Use ref. You did not measure sec met production (285-288)

AUTHOR REVISION: “In that day range, the maximum cellular devision occured. After carrying maximum cellular division, nutrients became limiting in the culture medium and the cell viability gradually decrease. This phase is called the stationary phase. The graph above shows that the stationary phase starts on the 17th day until 20th day. After the stasionary phase, the nutrient content in the medium became exhausted and toxic substances were produced by the cells (Bhojwani and Razdan 1983 in (Khanpour-Ardestani et al. 2015).”

68. How do you know. Please use ref (line 292)

AUTHOR REVISION: After the stasionary phase, the nutrient content in the medium became exhausted and toxic substances were produced by the cells (Bhojwani and Razdan 1983 in (Khanpour-Ardestani et al. 2015).”

69. So… what happen with Fig 6? Below you explain many things, but you did not explain what happen in lime cell culture after elicitation. Please elaborate use the Fig to explain more

AUTHOR REVISION: in this paragraf show cell line shape “During the incubation period, cells change in shape due to response to the environment and nutrient. The spherical shape indicates that the cell is the result of a previous cell division in *Stelechocarpus burahol* (BI.) Hook. F. and it is embryogenic cells with activated divisionin *Saccharum officinarum* LCells with a spherical shape will differentiate into elongated cells.In *Stelechocarpus burahol* (BI.) Hook. F., elongated cell indicated the non-viable condition and non-embriogenic cell showed in *Saccharum officinarum* L. (Habibah et al. 2017; Thorat et al. 2017). Acording to Ogita et al., 1997 in dos Santos et. al long binucleated cells undergo continuous cell division resulting in the development of adventitious somatic proembryos in *Larix leptolepis*(2010).

70. Not only environment, also nutrient, etc, please use more ref (line 298)

AUTHOR REVISION: in this paragraf show cell line shape “During the incubation period, cells change in shape due to response to the environment and nutrient. The spherical shape indicates that the cell is the result of a previous cell division in *Stelechocarpus burahol* (BI.) Hook. F. and it is embryogenic cells with activated divisionin *Saccharum officinarum* LCells with a spherical shape will differentiate into elongated cells.In *Stelechocarpus burahol* (BI.) Hook. F., elongated cell indicated the non-viable condition and non-embriogenic cell showed in *Saccharum officinarum* L. (Habibah et al. 2017; Thorat et al. 2017). Acording to Ogita et al., 1997 in dos Santos et. al long binucleated cells undergo continuous cell division resulting in the development of adventitious somatic proembryos in *Larix leptolepis*(2010).

71. Please use more ref examples of several plant cell culture (line 299)

AUTHOR REVISION: in this paragraf show cell line shape “During the incubation period, cells change in shape due to response to the environment and nutrient. The spherical shape indicates that the cell is the result of a previous cell division in

Stelechocarpus burahol (BI.) Hook. F. and it is embryogenic cells with activated divisionin *Saccharum officinarum* LCells with a spherical shape will differentiate into elongated cells.In *Stelechocarpus burahol* (BI.) Hook. F., elongated cell indicated the non-viable condition and non-embriogenic cell showed in *Saccharum officinarum* L. (Habibah et al. 2017; Thorat et al. 2017). Acording to Ogita et al., 1997 in dos Santos et. al long binucleated cells undergo continuous cell division resulting in the development of adventitious somatic proembryos in *Larix leptolepis*(2010).

72. More ref (303-304)

AUTHOR REVISION: in this paragraf show cell line shape "During the incubation period, cells change in shape due to response to the environment and nutrient. The spherical shape indicates that the cell is the result of a previous cell division in *Stelechocarpus burahol* (BI.) Hook. F. and it is embryogenic cells with activated divisionin *Saccharum officinarum* LCells with a spherical shape will differentiate into elongated cells.In *Stelechocarpus burahol* (BI.) Hook. F., elongated cell indicated the non-viable condition and non-embriogenic cell showed in *Saccharum officinarum* L. (Habibah et al. 2017; Thorat et al. 2017). Acording to Ogita et al., 1997 in dos Santos et. al long binucleated cells undergo continuous cell division resulting in the development of adventitious somatic proembryos in *Larix leptolepis*(2010).

73. Make 100 x magnification, clearer (mengomentari perbesaran figure 6.)

AUTHOR REVISION: We've edited figure 7 according to reviewer suggestion.

74. What is this all about, what the relation with your experiment? No data on sec met product of lime cell culture. Nothing to compare (line 313-327)

AUTHOR REVISION: these references show "Subculture of line cells with a certain period is one method to maintain cell viability to produce bioactive compounds." So in this experiment we try to determine growth of cell line for the future analyze.

75. How do you know? No data to show this. This paragraph is irrelevant. You can make any suggestion of your further research only. Please rewrite (line 331-332)

AUTHOR REVISION: these statement by Khanpour-Ardestani et al. (2015)

76. What is this? Ref? (line 334-336)

AUTHOR REVISION: "Subculture of line cells with a certain period is one method to maintain cell viability to produce bioactive compounds. According to a study conducted by Sierra et al. (1992) succeeded in stabilizing alkaloid compounds in two cell line cultures of the *Tabernaemontana divaricata*. The highest production of alkaloids in the 4th after the change of the medium and the growth remains stable during 30 subculture with a subculture interval of every 9 days. The other study was found in the production of betaxanthins in callus culture of *Beta vulgaris* L. var 'Dark Detroit'. Production of betaxanthins in callus cells line of the *Beta vulgaris* increased 1.8-fold after 48 subcultures

with subculture intervals every 14 days (Trejo-Tapia et al. 2008). As well as the stability of synthesizing verbascoside in suspension cell line culture of *Buddleja cordata* Kunth after being subcultured for 5 continuous years (Arano-Varela et al. 2020)"

77. This is incorrect, no data from this study (line 336-338)

    AUTHOR REVISION: "This study provided an efficient way to further regulation of biosynthesis and production of bioactive compounds on scale-up in kaffir lime cell line culture. Analyze post-subculture bioactive compounds and determine the appropriate subculture cycle to maximize the production of secondary metabolites warrant further investigation.

78. Need to recheck after the revision of Fig 5 (line 346-348)

    AUTHOR REVISION: revised figure 6 in manuscript

79. This paper is written by 7 authors. The data taken was limited. The contribution of authors is not significant. The output of 7 authors must have resulted in big and in detail data.

    D.Y.R. collected and analyzed the data and wrote the manuscript and revised it, F.D. collected and analyzed the data and wrote the manuscript, G.P.C wrote the manuscript, A.J.N. wrote the manuscript, A.B.S. analyzed the data, E.S. analyzed the data, W.A.S.T. design the research and supervised all process.